Sinus floor elevation surgery for enabling dental implant placement

Rickert, Daniela

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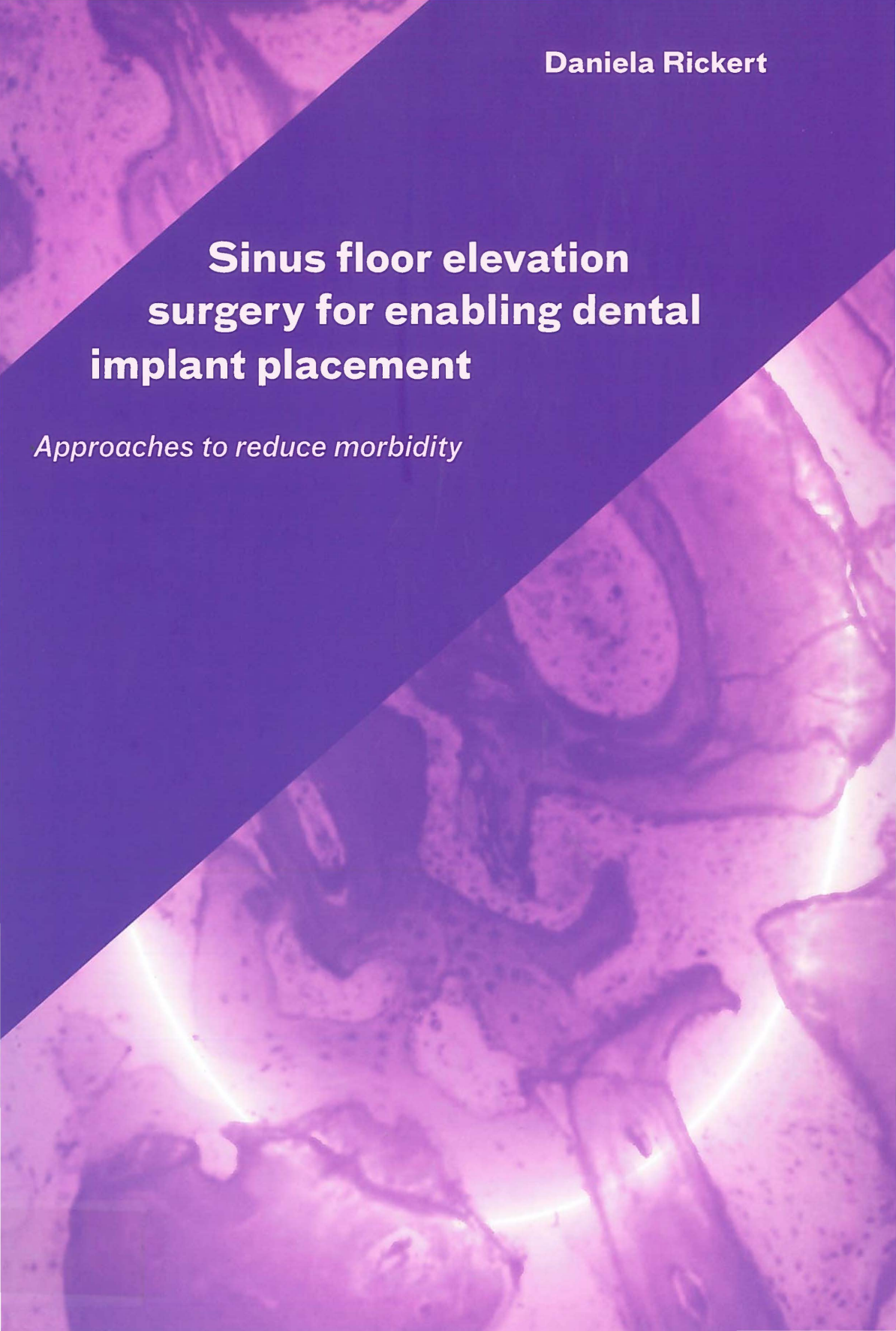
Approaches to reduce morbidity



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Stellingen behorende bij het proefschrift

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Daniela Rickert, 4 juli 2012.

1. Uit histologisch onderzoek blijkt er na een genezingsperiode van 3 maanden geen verschil te zijn in nieuwe botvorming tussen behandeling met BioOss® in combinatie met een beenmergconcentraat en BioOss® in combinatie met autoloog bot. *(dit proefschrift)*

2. Het percentage van implantaatverlies lijkt na een sinusbodemelevatie met BioOss® gemengd met een beenmergconcentraat hoger te zijn dan de percentages die binnen de implantologie doorgaans gehanteerd worden. *(dit proefschrift)*

3. In tegenstelling tot wat vaak wordt beweerd, is het risico op een perforatie van de membraan van de sinus maxillaris bij het gebruik van Piezochirurgie niet kleiner dan dat bij gebruik van conventioneel roterend instrumentarium. *(dit proefschrift)*

4. Het overlevingspercentage van implantaten is onafhankelijk van het gebruik van een botsubstituut, al dan niet in combinatie met autoloog bot of alleen autoloog bot voor het verhogen van de bodem van de sinus maxillaris, mits een voldoende lange genezingstijd in acht wordt genomen voordat de implantaten worden geplaatst. *(dit proefschrift)*

5. Proefschriften geven mensen geen wijsheid waar er geen wijsheid was, maar waar er wijsheid is, wordt deze door het lezen groter. *(vrij naar J. Harrington)*

6. Om een gezinsleven, een algemene tandartspraktijk en een promotietraject succesvol te kunnen combineren, moet regelmatig een tandje worden bijgezet.

7. Wetenschap is georganiseerde kennis. Wijsheid is georganiseerd leven. *(Citaat van I. Kant, bron: onbekend)*

8. De relatie tussen Duitsers en Nederlanders is onder meer afhankelijk van de voetbaluitslag Duitsland tegen Nederland.

9. Der Mensch muss bei dem Glauben verharren, dass das Unbegreifliche begreiflich sei, er würde sonst nicht forschen. *(J.W. von Goethe, Buch 2; Betrachtungen im Sinne der Wanderer.)*

10. Als we wisten wat we deden, heette het geen onderzoek. *(Citaat van A. Einstein, bron: onbekend)*

11. Een gepromoveerde tandarts heeft een kroon op zijn werk geplaatst.

CMB

RIJKSUNIVERSITEIT GRONINGEN

Sinus floor elevation surgery for enabling dental implant placement

Approaches to reduce morbidity

Proefschrift

ter verkrijging van het doctoraat in de
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door

Daniela Rickert

geboren op 11 september 1983
te Keulen, Duitsland

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Contents

Chapter 1

General Introduction

General Introduction

Application of dental implants to support prosthetic constructions has evolved into a viable alternative to conventional prosthetic procedures (1, 2). However, implant placement in the posterior maxilla often poses a problem due to an insufficient pre-existent bone volume (3, 4). An insufficient volume of bone to allow for reliable primary placement of implants is usually solved by a maxillary sinus floor elevation procedure using autogenous bone, bone substitutes or a mixture of autogenous bone and bone substitutes as grafting materials (2).

For sinus floor augmentations, autogenous bone is the most common used material and is currently still considered the golden standard, although numerous alternative materials have been used with variable results (4-6). Autogenous bone grafts are very popular because they have osteogenic, osteoinductive and/or osteoconductive properties, contain a high number of viable cells and are rich in growth factors. The viable cells consist of osteoblasts, undifferentiated mesenchymal cells, monocytes and osteoclast precursor cells. Bone substitutes do not provide the cellular elements necessary for osteogenesis and are considered to be just osteoconductive and need a longer healing time before implants can be placed (7). The various bonesubstitutes are either purely synthetic or most organic material has been removed from the substitute material, e.g., bovine bone, during the fabrication process.

Donor site morbidity is a problem accompanying harvesting techniques to obtain autogenous bone grafts. The donor site morbidity puts the patient at an inconvenience. This inconvenience probably can be reduced or even may be avoided when using bone substitutes; therefore the search for good substitutes continues (8). Thus, it can be questioned whether autogenous bone still should be considered as the grafting material of first choice in future studies. However, before bone substitutes can replace autogenous bone as the grafting material of first choice still a number of questions has to be solved regarding bone substitute as, amongst others, it still has to be settled what healing time has to be taken care of before placing implants in a site grafted with a bone substitute and whether a bone substitute can be solely applied or always has to be combined with autogenous bone. The same problem applies for the benefit of adding growth factors, e.g. in the form of platelet rich plasma to a grafting material. Results from the literature indicate that conclusions of whether a bone substitute or additive to a bone grafting material is worthwhile to be used are based on few trials, usually underpowered, having short follow-ups, and often judged to be at high risk of bias. Therefore, the various alternatives for autologous bone still should be viewed as preliminary and be interpreted with great caution. E.g., various authors studied or solely autogenous bone or only bone substitutes as a grafting material, but they did not compare the treatment outcome of various grafting materials with autogenous bone serving as a control (9-16).

Furthermore, many surgical techniques have been used to get access to the maxillary sinus via the lateral wall allowing for elevation of the sinus membrane, all with their own com-

plications and morbidity. Perforation of the Schneiderian membrane is the most common intraoperative complication of the various surgical approaches (17, 18). In most instances, perforation of the Schneiderian membrane occurs either while using rotative instruments to prepare the bone window or when using hand instruments to elevate the membrane from the sinus walls. In line with the tendency towards minimally invasive surgery, the use of ultrasonic waves for bone cutting has been introduced in oral and maxillofacial surgery. An important achievement of the latter approach, using a piezoelectric device, is the presumed lower risk on causing visible injury to the adjacent soft tissues and thus on perforating the sinus membrane (19, 20, 21). Again, sound studies to compare the efficacy of a piezoelectric device with a conventional approach to get access to the maxillary sinus are sparse in literature.

Finally, it recently has been shown in animal studies that seeding a bovine bone substitute (BioOss®) with a bone marrow concentrate rich in mononuclear stem cells (MSCs) may result in bone forming kinetics comparable to bone forming kinetics in a region solely reconstructed with autogenous bone (22). MSCs were shown to differentiate to osteoblasts when being introduced into an environment prone to formation of bone. In addition, in *in vitro* assays osteoblast-like cells were cultured on various alloplastic biomaterials used for reconstructive procedures in dental and craniomaxillofacial surgery (23). The latter study revealed that osteoblast like cells attach to BioOss® and offer suitable growth and proliferation conditions. Furthermore, Gutwald et al. (22) compared in a sheep model the osteogenic potential of mononuclear cells harvested from the iliac crest combined with bovine bone mineral to autogenous cancellous bone alone. Histomorphometric analysis of biopsies taken after 8 and 16 weeks after the sinus floor augmentation procedures revealed the bone forming potential of mononuclear cells, including MSCs, that were added to BioOss® as a biomaterial (23). Furthermore, Herten et al. (24) evaluated the influence of different bone substitutes (BioOss®) on the viability of human bone marrow MSCs *in vitro* and concluded that BioOss® supported cell viability and allowed for cell proliferation. As the results from *in vitro* and animal studies (22) were very promising, this approach has to be tested in human.

Aim of the thesis

The overall aim of this PhD study was to evaluate different approaches aiming for reduction of the morbidity accompanying sinus elevation surgery. The specific aims were:

- to systematically review the literature regarding the treatment outcome of residual maxillary ridges needing maxillary sinus floor elevation surgery to create a bone volume sufficient to allow for reliable implant placement in human. The objectives of this review were to assess the bone fraction and implant survival rate and to determine whether the bone fraction was affected by the grafting material or growth factor applied (**Chapter 2**).
- to assess the performance of conventional rotative instruments and a piezoelectric

device for maxillary sinus floor elevation surgery in reducing the morbidity of the sinus elevation surgery procedure in a randomized controlled trial (**Chapter 3**).

- to assess in a randomized controlled design whether differences in bone formation occur after maxillary sinus floor elevation surgery with either autogenous bone in combination with BioOss® or BioOss® seeded with a concentrate from posterior iliac crest bone marrow rich in MSCs (**Chapters 4** and **5**).
- to assess in a randomized controlled split mouth design the one-year implants survival rate, clinical and radiographic performance of the placed implants, and satisfaction of patients after maxillary sinus floor elevation surgery with BioOss® mixed with either autogenous bone or a bone marrow concentrate rich in MSCs (**Chapter 6**).

References

1. Visser A, Raghoebar GM, Meijer HJ, Vissink A. Implant-retained maxillary overdentures on milled bar suprastructures: a 10-year follow-up of surgical and prosthetic care and aftercare. Int J Prosthodont 2009: 22:181-192.

2. Slot W, Raghoebar GM, Vissink A, Huddleston Slater JJ, Meijer HJ. A systematic review of implant-supported maxillary overdentures after a mean observation period of at least 1 year. J Clin Periodontol 2010: 37: 98-110.

3. Ulm CW, Solar P, Gsellmann B, Matejka M, Watzek G. The edentulous maxillary alveolar process in the region of the maxillary sinus--a study of physical dimension. Int J Oral Maxillofac Implants 1995: 24: 279-282.

4. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 1998: 13: 11-45.

5. Hallman M & Thor A. Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. Periodontol 2000 2008: 47: 172-192.

6. Tong DC, Rioux K, Drangsholt M, Beirne OR. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. Int J Oral Maxillofac Implants 1998: 13: 175-182.

7. Thorwarth M, Srour S, Felszeghy E, Kessler P, Schultze-Mosgau S, Schlegel KA. Stability of autogenous bone grafts after sinus lift procedures: a comparative study between anterior and posterior aspects of the iliac crest and an intraoral donor site. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005: 100: 278-284.

8. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM, Burger EH. Histomorphometry of human sinus floor augmentation using a porous beta-tricalciumphosphate: a prospective study. Clin Oral Implants Res 2004: 15: 724-732.

9. Barone A, Crespi R, Aldini NN, Fini M, Giardino R, Covani U. Maxillary sinus augmentation: histologic and histomorphometric analysis. Int J Oral Maxillofac Implants 2005: 20: 519-525.

10. Bettega G, Brun JP, Boutonnat J, Cracowski JL, Quesada JL, Hegelhofer H, Drillat P, Richard MJ. Autologous platelet concentrates for bone graft enhancement in sinus lift procedure. Transfusion 2009: 49: 779-785.

11. Boyne PJ & James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980: 38: 613-616.

12. Cawood JJ & Howell RA. A classification of edentulous jaws. Int J Oral Maxillofac Surg 1988: 17: 232–236.

13. Consolo U, Zaffe D, Bertoldi C, Ceccherelli G. Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone. Clin Oral Implants Res 2007: 18: 252-262.

14. Daelemans P, Hermans M, Godet F, Malevez C. Autologous bone graft to augment the maxillary sinus in conjunction with immediate endosseous implants: a retrospective study up to 5 years. Int J Periodontics Restorative Dent 1997: 17: 27-39.

15. Del Fabbro M, Testori T, Francetti L, Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. Int J Periodontics Restorative Dent 2004: 24: 565-577.

16. Esposito M, Grusovin MG, Rees J, Karasoulos D, Felice P, Alissa R, Worthington H, Coulthard P. Effectiveness of sinus lift procedures for dental implant rehabilitation: a Cochrane systematic review. Eur J Oral Implantol 2010: 3: 7-26.

17. Schwartz-Arad D, Herzberg R, Dolev E. The prevalence of surgical complications of the sinus graft procedure and their impact on implant survival. J Periodontol 2004: 75: 511-516.

18. Pjetursson BE, Tan WC, Zwahlen M, Lang NP. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 2008: 35: 216-240.

19. Vercellotti T. Technological characteristics and clinical indications of piezoelectric bone surgery. Minerva Stomatol 2004: 53: 207-214.

20. Eggers G, Klein J, Blank J, Hassfeld S. Piezosurgery: an ultrasound device for cutting bone and its use and limitations in maxillofacial surgery. Br J Oral Maxillofac Surg 2004: 42: 451-453.

21. Wallace SS, Mazor Z, Froum SJ, Cho SC, Tarnow DP. Schneiderian membrane perforation rate during sinus elevation using piezosurgery: clinical results of 100 consecutive cases. Int J Periodontics Restorative Dent 2007: 27: 413-419.

22. Gutwald R, Haberstroh J, Kuschnierz J, Kister C, Lysek DA, Maglione M, Xavier SP, Oshima T, Schmelzeisen R, Sauerbier S.Mesenchymal stem cells and inorganic bovine bone mineral in sinus augmentation: Comparison with augmentation by autologous bone in adult sheep. Br J Oral Maxillofac Surg 2010: 48:285-290.

23. Schmitt SC, Wiedmann-Al-Ahmad M, Kuschnierz J, Al Ahmad A, Huebner U, Schmelzeisen R, Gutwald R. Comparative in vitro study of the proliferation and growth of ovine osteoblast-like cells on various alloplastic biomaterials manufactured for augmentation and reconstruction of tissue or bone defects. J Mater Sci Mater Med 2008: 19:1441-1450.

24. Herten M, Rothamel D, Schwarz F, Friesen K, Koegler G, Becker J. Surface- and nonsurface-dependent in vitro effects of bone substitutes on cell viability. Clin Oral Investig 2009:13:149-155.

Chapter 2

Maxillary sinus lift with solely autogenous bone compared to a combination of autogenous bone and growth factors or (solely) bone substitutes.

A systematic review.

This chapter is an edited version of the manuscript.

Rickert D, Huddleston Slater JJR, Meijer HJA, Vissink A, Raghoebar GM.
Int J Oral Maxillofac Surg. 2012: 41:160-167.

Abstract

Aim: To systematically review the literature regarding the treatment outcome of maxillary sinus floor elevation procedures used to create a sufficient bone volume to enable reliable implant placement. The objectives were to assess the bone volume and implant survival rate and to determine whether the bone volume is affected by the grafting material or growth factor applied.

Material and Methods: MEDLINE (1979– September 2010, via PUBMED) and EMBASE (1987- September 2010) were explored for trials in which sinus floor elevations with autogenous bone (control group) were compared with autogenous bone in combination with growth factors or bone substitutes, or solely with bone substitutes (test groups). Histomorphometric analysis was mandatory to compare results properly.

Results: Twelve out of 1124 selected studies fulfilled all inclusion criteria. Meta-analyses comparing the bone volume after applying autogenous bone, a combination of autologous bone with growth factors (platelet rich plasma), or a combination of autogenous bone and bone substitutes (bovine hydroxyapatite, bioactive glass, corticocancellous pig bone) revealed no significant differences in formation of new bone after a healing period of at least 5 months ($p=0.341$, $p=0.821$, $p=0.372$, $p=0.609$, respectively), while a significantly higher bone volume in the autogenous bone group was observed when compared to the sole use of β-tricalciumphosphate ($p=0.036$). The one-year overall implant survival rate showed no significant difference between implants placed in control or test sites (97.2% versus 98.2%, respectively).

Conclusion: It can be concluded that bone substitutes, such as bovine hydroxyapatite, bioactive glass or corticocancellous pig bone in combination with autogenous bone provide a reliable alternative for autogenous bone as a sole grafting material to reconstruct bony deficiencies in the maxillary sinus region, for supporting dental implants when allowing for an at least five months bone healing time. Addition of growth factors (platelet rich plasma) to a grafting material as well as the sole use of β-tricalciumphosphate did not promote the formation of new bone.

Introduction

Application of dental implants to support prosthetic constructions has evolved into a viable alternative to conventional prosthetic procedures. However, implant procedures in the posterior maxilla often pose a problem due to an insufficient pre-existent bone volume (1, 2). This restriction is not reserved to edentulous patients, but is also often observed in partial dentate patients needing an implant-based prosthodontic reconstruction in the posterior region of the maxilla. An insufficient bone level to allow for reliable primary placement of implants can be solved by a maxillary sinus floor elevation procedure using autogenous bone, bone substitutes or a mixture of autogenous bone and bone substitutes as grafting materials. Augmentation of the maxillary sinus floor with an autogenous bone graft, introduced by Boyne & James (3) and Tatum, Jr (4), is a commonly used method for increasing vertical bone height for insertion of dental implants.

During the maxillary sinus floor elevation procedure, the space created between the residual maxillary ridge and the elevated Schneiderian membrane is usually filled with a grafting material (5, 6). This way, a bone volume is created that may allow for reliable implant placement, either simultaneously with the elevation procedure when the residual ridge allows for primary implant stability or as a second stage after healing of the grafted site (1, 3, 7).

For sinus floor augmentations, autogenous bone is the most common used material and is still considered the gold standard (1, 8, 9), although numerous alternative materials have been used with variable results. Recent studies have demonstrated that the mere lifting of the sinus mucosal lining and simultaneous placement of implants also can result in bone formation without the use of a grafting material (10). However, currently this technique only is applied for conditions allowing for sufficient primary stability of implants during placement and a sufficient width of the alveolar crest but not for reconstruction in horizontal and vertical direction.

As mentioned in the previous paragraph, autogenous bone grafts are the most widely used bone grafts (11). Autografts are very popular because they have osteogenic, osteoinductive, osteoconductive properties, a high number of viable cells, and are rich in growth factors. The viable cells consist of osteoblasts, undifferentiated mesenchymal cells, monocytes and osteoclast precursor cells. These cells participate in the remodeling and formation of the new bone (8). Alternatives such as bone substitutes do not provide the cellular elements necessary for osteogenesis and are only osteoconductive (12). These alternatives are either synthetic or most organic material has been removed from the substitute material during the fabrication process.

Donor site morbidity is a problem accompanying bone-harvesting techniques and puts the patient at an inconvenience that probably can be reduced or even be avoided when using bone substitutes (13). Thus, it can be questioned whether autogenous bone should still be considered as the grafting material of first choice. In other words, can autogenous bone be (partially) replaced by bone-substitutes? Furthermore, it remains unclear what healing time has to be taken care of before placing implants in a site grafted with a bone substitute

(usually a site reconstructed with a bone substitute takes longer time before allowing for implant placement than a site reconstructed with autogenous bone) and whether a bone substitute can be solely applied or always has to be combined with autogenous bone. Moreover, as clinicians are keen for tools to speed up healing, the effect of using platelet rich plasma (PRP) has been studied on its presumed effect to speed bone regeneration. It has been speculated that growth factors that are present in platelet rich plasma could enhance healing of the grafts and might counteract resorption after augmentation (14).

The effect of maxillary sinus floor elevation on the survival of endosseous dental implants has been systematically reviewed in rather general terms in the past (3, 5, 15-23), but an in detail analysis of the efficacy of using an autogenous bone graft when compared with bone substitutes and bone growth factors on bone formation and implant was not executed. In their reviews, the various authors discussed or solely autogenous bone or only bone substitutes, but they did not compare the treatment outcome of various grafting materials with autogenous bone serving as a control. Furthermore, retrospective studies, case reports, prospective and also cohort studies were included for analysis in the reviews mentioned above. Thus, the conclusions of the various review papers were not based on the most reliable type of clinical studies. Also the consensus of the sixth European workshop on periodontology (24) emphasized the research need to answer comparative questions to establish the clinical benefit of bone augmentation with respect to alternative treatments and to compare different treatments in terms of among others effectiveness, adverse effects and morbidity. Finally, in a recent Cochrane review Esposito et al. (18) discussed the effectiveness of sinus lift procedures for dental implant rehabilitation. No statistically significant difference was observed for any of the evaluated interventions. Conclusions are based on few trials, usually underpowered, having short follow-ups, and often judged to be at high risk of bias, therefore they should be viewed as preliminary and interpreted with great caution. Furthermore, analysis of the efficacy of using an autogenous bone graft when compared with bone substitutes and bone growth factors on bone formation and implant survival was not studied.

Therefore, the aim of this study was to systematically review the literature regarding the treatment outcome of residual maxillary ridges needing maxillary sinus floor elevation surgery to create a sufficient bone volume enabling reliable implant placement in human. The objectives of this systematic review were to assess the bone volume and implant survival rate and to determine whether the bone volume is affected by the grafting material or growth factor applied.

Material and Methods

Search strategy

For this review, a thorough search of the literature was conducted in the electronic database of MEDLINE (1979- September 2010, via PUBMED) and EMBASE (1987- September 2010). Studies in which patients were treated with a maxillary sinus floor elevation with autogenous bone as a control group were searched. Types of intervention were shared in three subgroups; solely bone substitutes, autogenous bone in combination with bone substitutes, autogenous bone in combination with growth-factors. Outcome measures were bone volume after healing period and implant survival.

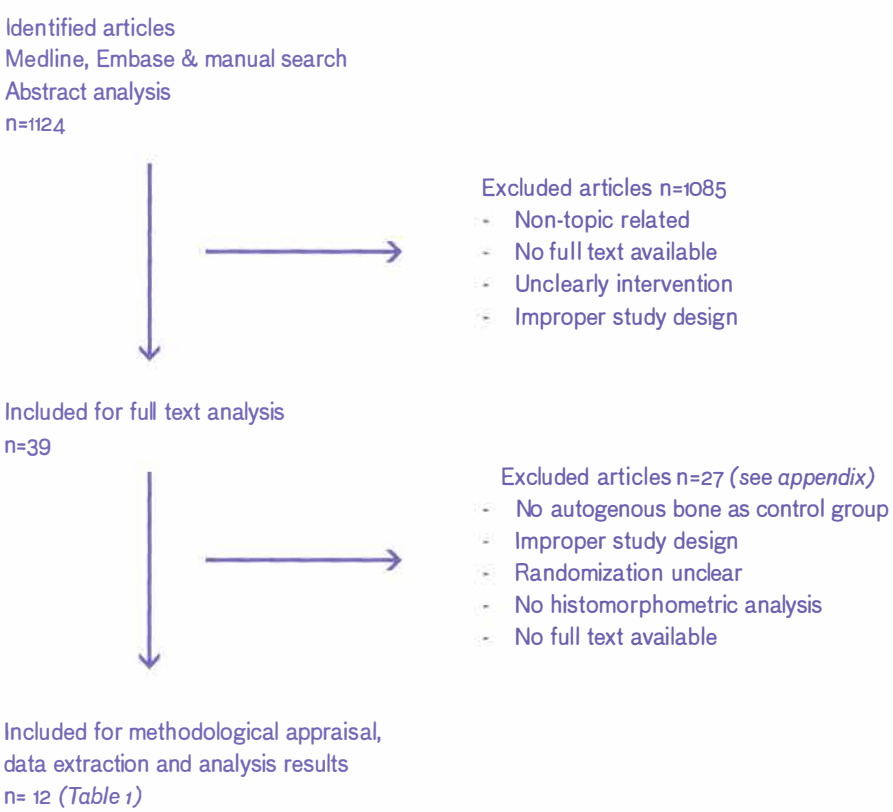
The search strategy used was a combination of MeSH terms and free text words; "Maxilllary sinus lift"[Mesh] OR (sinus augmentation) OR (sinus floor elevation) OR (maxillary sinus lift) OR (sinus graft) AND "Dental Prosthesis, Implant-Supported"[Mesh] OR "Implants, Experimental"[Mesh] OR "Prostheses and Implants"[Mesh] OR "Dental Implants"[Mesh] OR "Dental Implantation, Endosseous"[Mesh] OR "Dental Abutments"[Mesh] OR (alveolar atrophy) OR (implant*) OR (dental implant*) OR (oral implant*) AND (Humans[Mesh]). No language restrictions were applied.

Additionally, references of each included article of relevant review and eligible studies were checked. The titles and abstracts of the searches were assessed independently by two examiners. Full-text documents were obtained for possibly relevant articles. Manual searches of the bibliographies of all full text articles and related reviews selected from the electronic search were also performed and completed the revenue.

Studies

Longitudinal studies (randomized controlled trials (RCTs), clinical studies) were considered for evaluation. Patients with maxillary atrophy, who had undergone a maxillary sinus lift, could be included. It was chosen to include only studies in which autogenous bone was used as a control group as autogenous bone is considered the gold standard and is considered to be accompanied by the highest level of bone growth, at least during the first months after grafting. Moreover, including an autogenous bone group as a control also better allows for comparison of the various studies. Types of intervention were shared in three subgroups; solely bone substitutes, autogenous bone in combination with bone substitutes, autogenous bone in combination with growth-factors. Outcome measures were bone volume after healing period and implant survival after at least one year follow-up in patients with alveolar atrophy treated with a sinus lift procedure. This sinus lift was performed with autogenous bone alone (control group) compared to autogenous bone in combination with growth factors or bone substitutes, or solely with bone substitutes (test groups). Retrospective studies, studies with an improper study design, or an unclear intervention, case series, technical reports and reviews were excluded. Articles which were not topic related, with no full texts were excluded. Language was not restricted.

Figure 1. *Steps of the systematic review*



Quality assessment

Two reviewers independently assessed the methodological quality using the forms 'quality assessment of a cohort study' and 'quality assessment of a randomized clinical trial' developed by the Dutch Cochrane Centre, a centre of the Cochrane Collaboration (www.cochrane.nl). These two validity tools consist of 8 and 9 items, respectively, which have to be scored with a plus, minus or a question mark. It was decided that studies scoring four or more plusses were considered methodological acceptable. The two observers independently generated a score for the included articles. No blinding for author, institute or journal was performed. The main items of quality assessment were: Was the study randomized and the randomization procedure clearly stated? How good was the allocation concealment? Was a clear description of study group, inclusion and exclusion criteria, intervention and outcomes given? Was a clear description of withdrawals and drop outs given? Disagreements on validity assessment were resolved by discussion.

Data extraction and synthesis

For each study the following data were extracted and recorded in a data sheet:
- study design: randomized controlled trial (RCT) or prospective cohort study;
- treatment (control versus test);
- number of patients, type of patient (edentulous or not);
- details of type of intervention;
- number of sinus floor elevations, implants placed;
- details of the outcomes (new bone formation), implant survival;
- follow up time.

Statistical analysis

With respect to the quality assessment, agreement between the two reviewers regarding eligible studies was expressed as a percentage of agreement of Cohen's unweighted kappa. Failure rates were calculated by dividing the number of events (failures or complications) in the numerator by the total exposure time (implant time) in the denominator. The numerator was in all cases extracted directly from the publication or was provided by the authors of the original papers in cases in which only a part of the full sample was taken into consideration. The exposure time was extracted and calculated by multiplying the mean follow-up time by the number of implants available for the statistical analysis. The mean follow-up was directly extracted from the articles. For each study, event rates for implants were calculated by dividing the total number of events by the implants' exposure time in years. For further analysis, the total number of events was considered to be Poisson distributed (25). Univariate and multivariate Poisson regression analyses were performed using Stata®, version 10 SE (Stata Corp., College Station, TX, USA).
A meta-analysis was carried out for evaluating bone volume. Random effect models were created and a standardised weighted mean difference was used to evaluate bone volume. Meta-analysis was performed using the statistical software package 'Meta-analysis' (Comprehensive Meta-analysis Version 2.2, Biostat, Englewood NJ (2005), www.meta-analysis.com).

Results

The search in MEDLINE and EMBASE provided a total of 1124 articles reporting maxillary sinus floor elevation in combination with dental implant placement. Figure 1 outlines the study selection procedure. Articles which were not topic related, with no full texts were excluded. Also studies with an improper study design or an unclear intervention, case series, technical reports and reviews were excluded (n=1085). The κ-value for inter-reviewer agreement on the methodological appraisal was 0.85. Disagreement was generally caused by slight differences in interpretation and was easily resolved in a consensus discussion.
Finally, only 39 articles were selected for full-text analysis. Of these 39 articles, 27 further

Table 1. Included articles

Study	Study design	Control group	Test group	No of patients	No of elevations	No of implants	Biopsies taken after:	Edentulous/ dentate	Results (Bone volume) mean % ± SD	Follow-up	Implant survival rate
Bettega et al. (2009)	RCT split mouth	AB	AB + PRP	n=18	36	111	6 months	Both	co: 50 test: 43.2	12 months	co: 100% test: 100%
Schaaf et al. (2008)	RCT split mouth	AB	AB + PRP	n= 34*	68	?	4 months	Both	co: 35.3 ± 10.7 test: 33.3 ± 11.7	?	
Consolo et al. (2007)	RCT split mouth	AB	AB + PRP	n=16	32	?	4,5,6,7 months	Edentulous	co: 29.2 ± 4 test: 39.9 ± 5.7	?	
Suba et al. (2006)	RCT split mouth	AB	beta - TCP (Cerasorb)	n= 17	34	?	6 months	Edentulous	co: 34.7 ± 11.9 test: 32.4 ± 10.9	?	
Raghoebar et al. (2005)	RCT split mouth	AB	AB + PRP	n= 5	10	30	3 months	Edentulous	co: 41.1 ± 8.3 test: 38.4 ± 11.3	20.2 months	co: 100% test : 96.1%
Barone et al. (2005)	RCT split mouth	AB	AB + pig bone particles	n= 18	36	90	5 months	Both	co: 70 ± 19.9 test: 67 ± 14.9	?	
Szabó et al. (2005)	RCT split mouth	AB	β - TCP (Cerasorb)	n= 20	40	80	6 months	Edentulous	co: 38.8 ± 7.4 test: 36.5 ± 6.9	6 months	co: 95.1% test: 95.1%

Table 1. Included articles (continued)

Study	Study design	Control group	Test group	No of patients	No of elevations	No of implants	Biopsies taken after:	Edentulous/ dentate	Results (Bone volume) mean % ± SD	Follow-up	Implant survival rate
Zijderveld et al. (2005)	RCT split mouth	AB	β - TCP (Cerasorb)	n= 6*	12	31	6 months	Both	co: 41 ± 10 test: 19.2 ± 5.2	11 months	co: 100% test: 100%
Zerbo et al. (2004)	RCT split mouth	AB	β - TCP (Cerasorb)	n= 5*	10	?	5 months	Both	co: 41 ± 10 test: 19 ± 5	?	co: 100% test: 100%
Turunen et al. (2004)	RCT split mouth	AB	AB + BG particles	n=17	34	?	4 months	Both	co: 25.1 ± 7.1 test: 25.7 ± 7.4	17 months	
Hallman et al. (2002)	RCT split mouth	AB	AB + Bio-Oss 20% - 80%	n= 11*	22	68	6 months	Both	co: 37.7± 31.3 test: 39.9 ± 8	12 months	co: 83.3% test: 94.4%
Tadjoedin et al. (2000)	RCT split mouth	AB	AB + BG particles	n= 10	20	72	4,5,6 months	Edentulous	co: 42.2 ± 4.5 test: 34.5 ± 1.6	?	?

RCT= randomized clinical trial
AB= autogenous bone
PRP= platelet rich plasma,β- TCP= β-tricalciumphosphates, BG= biactive glass
co= control group, test= test group
?= no implants placed, therefore also no implant success rate, no follow-up time
* Only results of patients treated with split mouth design are used.

articles had to be excluded because they did not satisfy the inclusion criteria (see the appendix available online). These articles were excluded due to improper study design (not longitudinal, not prospective or unclear description of randomization), for not executing histomorphometric analysis or for not including autogenous bone as control group (details of the excluded studies are given in the online appendix). Authors who did not describe randomization clearly were contacted via e-mail for additional information. When a proper randomization procedure was applied, these studies were added to the results (14, 22, 26, 27). All 12 articles that fulfilled the inclusion criteria were randomized clinical trials (RCTs), and it worked out that all studies had applied a split mouth design. There were considerable differences in the selected articles regarding the number of patients, residual bone height, graft materials used, whether implants were placed or not and follow up. Furthermore, there were some differences in data reporting and in inclusion and exclusion criteria used in the various studies.

Description of studies

In all eligible maxillary sinus floor elevation studies autogenous bone was used as a control group. Types of intervention were shared in three subgroups; solely bone substitutes, autogenous bone in combination with bone substitutes, autogenous bone in combination with growth-factors. Outcome measures were bone volume after healing period and implant survival.

In the various included studies, patients had been treated with autogenous bone, autogenous bone in combination with bone substitutes or PRP (Table 1). In five out of twelve studies patients were edentulous, in the others edentulous and dentate patients were treated. In five publications the number of implants placed, the implant survival rate and follow up was mentioned. For an overall survival rate a comprehensive meta- analysis of these five studies was performed (Table 2).

The morphometric methods for analyzing the biopsies used in the included studies were comparable. Quantitative and qualitative investigations of the biopsies were performed with light microscopy and a computerized image analysis system was applied to analyze in the histological sections.

Histomorphometric results

Quantitative data analysis (bone volume) of the data provided in the twelve included studies was executed. Bone volume was defined as the percentage of the total bone volume. In the various meta-analyses performed, results five months after sinus floor augmentation were used because most included studies showed histomorphometric results after 5-6 months.

Platelet-rich plasma (PRP). Consolo et al. (17) showed a significant difference in bone volume in areas reconstructed with a combination of autogenous bone and PRP (39.9%) or solely with autogenous bone (29.2%) at four and five months after sinus floor eleva-

tion. Biopsies taken after six and seven months did not show any statistical difference. By contrast, Raghoebar et al. (7), Bettega et al. (15) and Schaaf et al. (28) did not observe any significant difference between both treatments, both at the three and six months evaluations. A meta-analysis of the four included articles failed to show a significant effect of PRP (p=0.341).

β-tricalciumphosphate. β-tricalciumphosphate (Cerasorb) has been used in the intervention groups in the studies of Zerbo et al. (13), Suba et al. (29), Szabo et al. (30) and Zijderveld et al (31). A meta-analysis of these revealed a significantly higher bone volume 5-6 months after treatment when applying autogenous bone (p=0.036).In the controls, augmented with autogenous bone, the newly formed bone was mostly lamellar, mature bone (80%). In the β-tricalciumphosphate sites, the newly formed bone was at comparable time points more immature and had a predominantly woven character (74%) (13).

Bioactive glass. Regarding bioactive glass Tadjoedin et al. (27) showed that augmentation with autogenous bone had resulted in a significant higher bone volume (42.2%) than treatment with bioactive glass (34.5%) four and five months after sinus floor elevation. However, Turunen et al. (32) presented in his study comparable results for both groups after 4 months of healing time. In the control group 25% of new bone was seen and in the test group 26%. A meta-analysis of the included studies showed no differences (p=0.372), thus treatment with autogenous bone alone was shown to be as good as treatment with a combination from autogenous bone and bioactive glass when allowing for a 5 months healing time.

Pig bone. Barone et al. (14) compared in his study autogenous bone with a combination of autogenous bone and pig bone particles. Also he did not show any significant differences five months after treatment with autogenous bone alone or in combination with pig bone particles when allowing for a 5 months healing time (control 70%± 19.9, test 67%±14.9).

Bio-Oss®. Hallman et al. (26) used autogenous bone in combination with Bio-Oss® (20%-80%) in the test group and showed comparable results in both groups six months after sinus floor elevation. The corresponding values for the bone volume area parameter were 37.7%±31.3 (control group) and 39.9%±8 (test group). Furthermore a third treatment group was composed of patients who accepted the treatment with a two stage sinus lift with 100% Bio-Oss®. The mean healing time of this group was prolonged to an average of 8.5 months because the newly formed bone was too immature after six months to provide enough primary stability for dental implant placement. In this group bone volume was 41.7%±26.6, after a healing time of 8.5 months. Results of this group are not included in the analysis because it was not compared with an autogenous bone group.

Implant survival

Implants inserted in grafts either composed of bone substitutes alone, bone substitutes combined with growth factors or a mixture of autogenous bone and substitutes, all achieved a one-year survival rate as high as implants placed in autogenous bone alone (Tables 1 and 2). The overall implant survival rate from these studies was 97.2 % for the control group treated

Table 2. Meta-analysis of implant survival rate of included studies.

Study	Intervention	Number of implants	Follow- up (Months)	Exposure Time (Years and months)	Failures	Failure rate in %	SE_Failure rate in %	Survival rate 1 year in %
Bettega et al. (2009)	AB	55	12	55 years and 0 months	0	0	0	100
Raghoebar et al. (2005)	AB	15	20.2	25 years and 3 months	0	0	0	100
Szabo et al. (2005)	AB	40	6	20 years and 0 months	1	5	5	95.1
Zijderveld et al. (2005)	AB	16	11	14 years and 5 months	0	0	0	100
Hallman et al. (2002)	AB	33	12	33 years and 0 months	6	18	7.4	83.4
Fixed						2.8	1.8	97.2
Bettega et al. (2009)	AB+ PRP	56	12	56 years and 0 months	0	0	0	100
Raghoebar et al. (2005)	AB+PRP	15	20.2	25 years and 3 months	1	4	4	96.1
Szabo et al. (2005)	beta-TCP	40	6	20 years and 0 months	1	5	5	95.1
Zijderveld et al. (2005)	beta-TCP	15	11	13 years and 6 months	0	0	0	100
Hallman et al. (2002)	AB+ Bio-Oss	35	12	35 years and 0 months	2	5.7	4	94.5
Fixed						1.8	1.1	98.2

AB= autogenous bone; PRP= platelet rich plasma, β-TCP= β-tricalciumphosphate; SE= standard error; Exposure time=calculated by multiplying the mean follow-up time by the number of implants available

with autogenous bone alone and 98.2 % for the various test groups (Table 2). Implant survival was defined as the percentage of implants initially placed that was still present at follow-up, one year after surgery of implant placement and is calculated as 1 – event rate.

Discussion

From this systematic review of the literature, evaluating studies in which the bone volume after sinus floor elevation surgery was evaluated by histomorphological analysis, it is obvious that adequate bone formation in a created space (e.g. the space created between the residual maxillary ridge and the elevated Schneiderian membrane) can be achieved with a variety of materials when a reasonable healing period (5-6 months) has been allowed for. Moreover, according to the findings of the present study, there is no clinical evidence for superiority of autogenous bone grafts above most bone substitutes in sinus floor elevation procedures when allowing for such a healing period. Furthermore, as the iliac crest is commonly used as donor site for patients who need a bilateral, vertical maxillary sinus lift, replacement of autogenous bone by bone substitutes might decrease the morbidity and discomfort of the grafting procedure from perspective of the patient.

Histomorphometry

In this study, autogenous bone served as a control group. Moreover, it has been shown that the bone volume measured in area grafted with autogenous bone was comparable to that grafted with (a mixture of autogenous bone and) bone substitutes when allowing for a reasonably long healing period of at least 5 months (26). However, it has to be mentioned that a comparable bone volume is also present in areas grafted with just autogenous bone after shorter healing periods (3-4 months; (33)) thus allowing for earlier implant placement in such sites.
It has been speculated that growth factors that are present in PRP could enhance healing of the grafts and also counteract resorption after augmentation (34). However, various authors concluded that no relevant differences in healing of soft tissues and bone existed between sites reconstructed with autogenous bone and autogenous bone mixed with PRP (7, 15, 28). Four trials evaluated the possible advantage of using PRP to accelerate bone healing for sinus augmentation (7, 15, 17, 28). No clinical benefit could be observed in a meta-analysis of these studies when using PRP, in other words there is no scientific support for justifying use in application.
Meta-analyses comparing the bone volume after applying β-tricalciumphosphate revealed a significantly higher bone volume 5-6 months after treatment when applying autogenous bone ($p=0.036$). Szabo et al. (30) showed after a 6 months healing period comparable results for both groups. However, Zerbo et al. (13) and Zijderveld et al. (31) concluded that in the controls, augmented with autogenous bone, the newly formed bone was significantly higher than in the β-tricalciumphosphate group after the same healing period.

Bioactive glass, a material that has been shown to be able to directly chemically bond to bone, has been shown a potentially applicable grafting material for reconstructive procedures. When applied in the size range of 300 to 355 µm, bioactive glass showed osteoconductive properties (27, 35). Turunen et al. (32) showed that the combined use of bioactive glass granules with autogenous bone chips for augmentation of the maxillary sinus floor diminished the amount of bone needed for augmentation and resulted in the same quantity of bone as when just autogenous bone chips were used. However, Tadjoedin et al. (27) showed in the control group, using bioactive glass particles in combination with autogenous bone, that new bone formation increased rapidly within two months, from 28.5% at four months to 38.1% at six months. Thus, as the healing period is sufficiently long (5 months), there are no differences in treatment and bioactive glass particles seem to be a good alternative for autogenous bone.

Barone et al. (14) compared in his study autogenous bone with a combination of autogenous bone and pig bone particles. In his study no significant differences, five months after treatment with autogenous bone alone or in combination with pig bone particles were shown when allowing for a 5 months healing time.

The use of bovine bone (Bio-Oss®) in combination with autogenous bone offers many additional advantages. First, it allows the volume of the graft to be at least doubled, avoiding the need to harvest large amounts of autogenous bone. This advantage might also apply to other substitutes. Second, the osteoconductive properties of bovine bone act as a scaffold that is essential for bone remodeling. Third, bovine bone is a calcium-deficient carbonate apatite with a crystal size of approximately 10 nm. Therefore, the surface area of each graft particle is considerably greater than that of porous bioceramics, making its resorption considerably slower (36). In addition, Hallman et al. (26) concluded from their clinical and histological study that similar short-term results can be expected when using autogenous bone, bovine hydroxyapatite, or a mixture of them for maxillary sinus floor augmentation and delayed placement of dental implants.

Implant survival

In table 2 survival rate of implants is shown. Implant survival rate was defined as the percentage of implants that was present at one year follow-up after insertion of the implants. In some studies, no implants were placed, neither did the authors report on the implant survival rate or the follow up of the studies was less than 1 year. The available data point towards a comparable implant survival in areas reconstructed with either autogenous bone alone or bone subsitutes in combination with autogenous bone. This is also supported by the results from the study by Nkenke & Stelzle et al. (21). When using autogenous bone alone the healing period can be reduced to average three months, whereas it is at least five months when using a bone substitute either alone or in combination with autogenous bone. A number of systematic reviews and meta-analysis has been performed on studies in which patients underwent a sinus floor elevation. For example, the objective of the study

from Graziani et al. (19) was to review implant survival following sinus floor augmentation procedures with conventional implant placement in the posterior maxilla. This systematic review suggests that implant survival in the augmented maxillary sinus is more variable (36%-100%) than that of implants placed in the posterior maxilla (73-100%). This study is restricted because of the limited data from controlled trails comparing implant survival in the augmented maxillary sinus and the posterior maxilla. Pjetursson et al. (37) studied the surface of implants and its failure rate. The best results were obtained using rough surface implants (98.3% implant survival after 3 years). The analysis in the study from Nkenke and Stelzle (21) focused on the question if autogenous bone is superior to bone substitutes. This study was limited to titanium implants with modified surfaces placed in sites with 6mm of residual bone height. Therefore, the retrieved evidence provides a low level of support for selection of autogenous bone or bone substitutes.

Quality assessment

The various reviews yet published in the international literature did not have such strict inclusion criteria as the current review (5, 15-23, 37). In the current systematic review methodological quality was assessed using specific study-design related forms designed by the Dutch Cochrane Collaboration. The results of the previous systematic reviews are limited because no well designed studies were included for analysis, furthermore no autogenous bone group as control group and/or no split mouth design was generally used to compare different outcomes and not always healing time was taken into account. Thus no homogeneity between the studies can be reached limiting the analysis of the data.

Limitations

A limitation of this systematic review might be, that studies comparing various types of bone substitutes, but not having included autogenous bone as a control, were not included as the outcome of these studies might be biased in showing a higher formation of bone with one substitute that even might surpass the level of bone growth as observed in other studies by autogenous bone alone. However, when autogenous bone would have been applied in those studies, it might have been the case that also in that study autogenous bone would have performed better than the substitutes studied. Furthermore, histomorphometric analysis was required to allow for comparison of results.

In fact, restrictions of the present review, multiple confounding variables such as type of implant, membrane application, height of the residual bone, timing of implant placement, patients compliance and habits might have influenced the outcomes (1).

In future, innovative techniques to promote bone healing to induct growth of bone will be introduced. To reconstruct bony defects, for example, adding mononuclear stem cells derived from a bone marrow aspirate to Bio-Oss® has been shown to result in bone forming kinetics comparable to autogenous bone alone. This after a healing period of three to four

months (33, 38). These mononuclear stem cells can differentiate to osteoblasts if they are added to a bony matrix. Moreover, the use of a grafting material to perform a maxillary sinus floor elevation procedure may become questionable as a recent study has demonstrated that the mere lifting of the sinus mucosal lining and simultaneous placement of implants also can result in bone formation (10). However, currently this technique only is applied for conditions allowing for sufficient primary stability of implants during placement and a sufficient width of the alveolar crest and not for bigger reconstruction in horizontal or vertical direction. Moreover, for evidence whether this treatment indeed reliably will result in induction of bone growth, well designed studies have to be carried out.

Conclusion

Taking these limitations into account, it can be concluded from the present systematic review of the literature that bone substitutes, such as Bio-Oss®, bioactive glass or cortico-cancellous pig bone in combination with autogenous bone, form an alternative for autogenous bone alone to reconstruct bony deficiencies in the maxillary sinus region, for supporting dental implants when taking a healing period of at least five months into account. When applying ß-tricalciumphosphate, the use of a mixture with autogenous bone is preferred to allow for a not too long healing period before implant placement. Furthermore, there is no scientific support for adding PRP to autogenous bone or bone substitutes to speed up the healing time of bone. Finally, short-term implant survival, the major clinical outcome, is not influenced by the various grafting procedures applied when allowing for a sufficient healing time before implant placement.

References

1. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 1998:13: 11-45.

2. Ulm CW, Solar P, Gsellmann B, Matejka M, Watzek G. The edentulous maxillary alveolar process in the region of the maxillary sinus--a study of physical dimension. Int J Oral Maxillofac Implants 1995: 24: 279-282.

3. Boyne PJ & James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980: 38: 613-616.

4. Tatum H, Jr. Maxillary and sinus implant reconstructions. Dent Clin North Am 1986: 30: 207-229.

5. Del Fabbro M, Testori T, Francetti L, Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. Int J Periodontics Restorative Dent2004:24: 565-577.

6. Wallace SS, Froum SJ, Cho SC, Elian N, Monteiro D, Kim BS, Tarnow DP. Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: histomorphometric and clinical analyses. Int J Periodontics Restorative Dent 2005: 25: 551-559.

7. Raghoebar GM, Schortinghuis J, Liem RS, Ruben JL, van der Wal JE, Vissink A. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clin Oral Implants Res 2005: 16: 349-356.

8. Hallman M & Thor A. Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. Periodontol 2000 2008: 47: 172-192.

9. Tong DC, Rioux K, Drangsholt M, Beirne OR. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. Int J Oral Maxillofac Implants 1998:13: 175-182.

10. Lundgren S, Cricchio G, Palma VC, Salata LA, Sennerby L. Sinus membrane elevation and simultaneous insertion of dental implants: a new surgical technique in maxillary sinus floor augmentation. Periodontol 2000 2008: 47: 193-205.

11. Daelemans P, Hermans M, Godet F, Malevez C. Autologous bone graft to augment the maxillary sinus in conjunction with immediate endosseous implants: a retrospective study up to 5 years. Int J Periodontics Restorative Dent 1997:17: 27-39.

12. Thorwarth M, Srour S, Felszeghy E, Kessler P, Schultze-Mosgau S, Schlegel KA. Stability of autogenous bone grafts after sinus lift procedures: a comparative study between anterior and posterior aspects of the iliac crest and an intraoral donor site. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005: 100: 278-284.

13. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM, Burger EH. Histomorphometry of human sinus floor augmentation using a porous beta-tricalciumphosphate: a prospective study. Clin Oral Implants Res 2004: 15: 724-732.

14. Barone A, Crespi R, Aldini NN, Fini M, Giardino R, Covani U. Maxillary sinus augmentation: histologic and histomorphometric analysis. Int J Oral Maxillofac Implants 2005: 20: 519-525.

15. Bettega G, Brun JP, Boutonnat J, Cracowski JL, Quesada JL, Hegelhofer H, Drillat P, Richard MJ. Autologous platelet concentrates for bone graft enhancement in sinus lift procedure. Transfusion 2009: 49: 779-785.

16. Cawood JJ & Howell RA. A classification of edentulous jaws. Int J Oral Maxillofac Surg 1988:17: 232–236.

17. Consolo U, Zaffe D, Bertoldi C, Ceccherelli G. Platelet-rich plasma activity on maxillary sinus floor augmentation by autologous bone. Clin Oral Implants Res 2007: 18: 252-262.

18. Esposito M, Grusovin MG, Rees J, Karasoulos D, Felice P, Alissa R, Worthington H, Coulthard P. Effectiveness of sinus lift procedures for dental implant rehabilitation: a Cochrane systematic review. Eur J Oral Implantol 2010: 3: 7-26.

19. Graziani F, Donos N, Needleman I, Gabriele M, Tonetti M. Comparison of implant survival following sinus floor augmentation procedures with implants placed in pristine posterior maxillary bone: a systematic review. Clin Oral Implants Res 2004:15: 677-682.

20. Klijn RJ, Meijer GJ, Bronkhorst EM, Jansen JA. Sinus Floor A Meta-Analysis of Histomorphometric Results and Graft Healing Time of Various Biomaterials Compared to Autologous Bone Used as Sinus Floor Augmentation Material in Humans. Tissue Eng Part B 2010: 16: 493-507.

21. Nkenke E & Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clin Oral Implants Res 2009:20: 124-133.

22. Plachokova AS, Nikolidakis D, Mulder J, Jansen JA, Creugers NH. Effect of platelet-rich plasma on bone regeneration in dentistry: a systematic review. Clin Oral Implants Res 2008: 19: 539-545.

23. Wallace SS & Froum SJ. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Ann Periodontol 2003: 8: 328-343.

24. Tonetti MS & Hammerle CH. Advances in bone augmentation to enable dental implant placement: Consensus Report of the Sixth European Workshop on Periodontology. J Clin Periodontol 2008: 35: 168-172.

25. Kirkwood BR & Sterne JAC. Essential Medical Statistics. Poisson Regression. Oxford: Blackwell Science Ltd. 2003: Chapter 24.

26. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. Int J Oral Maxillofac Implants 2002:17: 635-643.

27. Tadjoedin ES, de Lange GL, Holzmann PJ, Kulper L, Burger EH. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. Clin Oral Implants Res 2000: 1: 334-344.

28. Schaaf H, Streckbein P, Lendeckel S, Heidinger K, Gortz, B, Bein G, Boedeker RH, Schlegel KA, Howaldt HP. Topical use of platelet-rich plasma to influence bone volume in maxillary augmentation: a prospective randomized trial. Vox Sang 2008: 94: 64-69.

29. Suba Z, Takacs D, Matusovits D, Barabas J, Fazekas A, Szabo G. Maxillary sinus floor grafting with beta-tricalciumphosphate in humans: density and microarchitecture of the newly formed bone. Clin Oral Implants 2006: 17: 102-108.

30. Szabo G, Huys L, Coulthard P, Maiorana C, Garagiola U, Barabas J, Nemeth Z, Hrabak K, Suba Z. A prospective multicenter randomized clinical trial of autogenous bone versus beta-tricalciumphosphate graft alone for bilateral sinus elevation: histologic and histomorphometric evaluation. Int J Oral Maxillofac Implants 2005:20: 371-381.

31. Zijderveld SA, Zerbo IR, van den Bergh JP, Schulten EA, ten Bruggenkate CM. Maxillary sinus floor augmentation using a beta-tricalciumphosphate (Cerasorb) alone compared to autogenous bone grafts. Int J Oral Maxillofac Implants 2005:20: 432-440.

32. Turunen T, Peltola J, Yli-Urpo A, Happonen RP. Bioactive glass granules as a bone adjunctive material in maxillary sinus floor augmentation. Clin Oral Implants Res 2004:15: 135-141.

33. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoebar GM. Maxillary sinus floor elevation with Bio-Oss® combined with either autogenous bone or autogenous stem cells. A prospective randomized clinical trial. Clin Oral Implants Res 2011: 22: 251-258.

34. Thor A, Franke-Stenport V, Johansson CB, Rasmusson L. Early bone formation in human bone grafts treated with platelet-rich plasma: preliminary histomorphometric results. J Oral Maxillofac Surg 2007:36: 1164-1171.

35. Tadjoedin ES, de Lange GL, Lyaruu DM, Kuiper L, Burger EH. High concentrations of bioactive glass material (BioGran) vs. autogenous bone for sinus floor elevation. Clin Oral Implants Res 2002:13: 428-436.

36. Galindo-Moreno P, Avila G, Fernandez-Barbero JE, Aguilar M, Sanchez-Fernandez E, Cutando A, Wang HL. Evaluation of sinus floor elevation using a composite bone graft mixture. Clin Oral Implants Res 2007:18: 376-382.

37. Pjetursson BE, Tan WC, Zwahlen M, Lang NP. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 2008: 35: 216-240.

38. Schmitt SC, Wiedmann-Al-Ahmad M, Kuschnierz J, Al Ahmad A, Huebner U, Schmelzeisen R, Gutwald R. Comparative in vitro study of the proliferation and growth of ovine osteoblast-like cells on various alloplastic biomaterials manufactured for augmentation and reconstruction of tissue or bone defects. J Mater Sci Mater Med 2008: 19: 1441-1450.

Appendix

	Study	Reason(s) for exclusion
1	Becktor et al. (3)	No autogenous bone group/case series
2	Lee et al. (11)	No autogenous bone group
3	Knabe et al. (10)	No autogenous bone group
4	Mangano et al. (13)	No autogenous bone group
5	Crespi et al. (5)	Retrospective study
6	Schwartz et al. (18)	No autogenous bone group
7	Zizelmann et al. (27)	Improper design/No randomization
8	Thor et al. (23)	No randomization→ left/right
9	Nedir et al. (14)	Pilot study
10	Schlegel et al. (17)	No autogenous bone group/case series
11	Scarano et al. (16)	Improper design/ Unclear randomization
12	Serra E Silva FM et al. (19)	Unclear results
13	Choukroun et al. (4)	No autogenous bone group
14	Wallace et al. (25)	Not available
15	Thor et al. (22)	No histomorphometric analysis
16	Steigmann & Garg (20)	No autogenous bone group
17	Noumbissi et al. (15)	Not available
18	Thorwarth et al. (24)	No test group
19	Artzi et al. (2)	No autogenous bone group/ unclear rand.
20	Degidi et al. (6)	Not available
21	John & Wenz (8)	Improper design/ Unclear randomization
22	Wiltfang et al. (26)	No autogenous bone group
23	Kasabah et al. (9)	No autogenous bone group
24	Tadjoedin et al. (21)	Improper design/Unclear randomization
25	Artzi et al. (1)	No autogenous bone group
26	Lorenzetti et al. (12)	Improper design/Unclear randomization
27	Jensen & Sindet-Pedersen (7)	Case reports

References (Appendix)

1. Artzi Z, Nemcovsky CE, Tal H, Dayan D. Histopathological morphometric evaluation of 2 different hydroxyapatite-bone derivatives in sinus augmentation procedures: a comparative study in humans. J Periodontol 2001: 72: 911-920.

2. Artzi Z, Kozlovsky A, Nemcovsky CE, Weinreb M. The amount of newly formed bone in sinus grafting procedures depends on tissue depth as well as the type and residual amount of the grafted material. J Clin Periodontol: 32:193-199.

3. Becktor JP, Hallstrom H, Isaksson S, Sennerby L. The use of particulate bone grafts from the mandible for maxillary sinus floor augmentation before placement of surface-modified implants: results from bone grafting to delivery of the final fixed prosthesis. J Oral Maxillofac Surg 2008: 66: 780-786.

4. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan, AJ, Mouhyi J, Dohan DM. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006:101: 299-303.

5. Crespi R, Vinci R, Cappare P, Gherlone E, Romanos GE. Calvarial versus iliac crest for autologous bone graft material for a sinus lift procedure: a histomorphometric study. Int J Oral Maxillofac Implants 2007:22: 527-532.

6. Degidi M, Piattelli M, Scarano A, Iezzi G, Piattelli A. Maxillary sinus augmentation with a synthetic cell-binding peptide: histological and histomorphometrical results in humans. J Oral Implantol 2004: 30: 376-383.

7. Jensen J & Sindet-Pedersen S. Autogenous mandibular bone grafts and osseointegrated implants for reconstruction of the severely atrophied maxilla: a preliminary report. J Oral Maxillofac Surg 1991:49: 1277-1287.

8. John HD & Wenz B. Histomorphometric analysis of natural bone mineral for maxillary sinus augmentation. Int J Oral Maxillofac Implants 2004: 19: 199-207.

9. Kasabah S, Simunek A, Krug J, Lecaro MC. Maxillary sinus augmentation with deproteinized bovine bone (Bio-Oss) and Impladent dental implant system. Part II. Evaluation of deprotienized bovine bone (Bio-Oss) and implant surface. Acta Medica (Hradec Kralove) 2002: 45: 167-171.

10. Knabe C, Koch C, Rack A, Stiller M. Effect of beta-tricalciumphosphate particles with varying porosity on osteogenesis after sinus floor augmentation in humans. Biomaterials 2008: 29: 2249-2258.

11. Lee CY, Rohrer MD, Prasad HS. Immediate loading of the grafted maxillary sinus using platelet rich plasma and autogenous bone: a preliminary study with histologic and histomorphometric analysis. Implant Dent: 17: 59-73.

12. Lorenzetti M, Mozzati M, Campanino PP, Valente G. Bone augmentation of the inferior floor of the maxillary sinus with autogenous bone or composite bone grafts: a histologic-histomorphometric preliminary report. Int J Oral Maxillofac Implants 1998:13: 69-76.

13. Mangano C, Scarano A, Perrotti V, Iezzi G, Piattelli A. Maxillary sinus augmentation with a porous synthetic hydroxyapatite and bovine-derived hydroxyapatite: a comparative clinical and histologic study. Int J Oral Maxillofac Implants 2007: 22: 980-986.

14. Nedir R, Bischof M, Vazquez L, Szmukler-Moncler S, Bernard JP. Osteotome sinus floor elevation without grafting material: a 1-year prospective pilot study with ITI implants. Clin Oral Implants Res 2006: 17: 679-686.

15. Noumbissi SS, Lozada JL, Boyne PJ, Rohrer MD, Clem D, Kim JS, Prasad H. Clinical, histologic, and histomorphometric evaluation of mineralized solvent-dehydrated bone allograf (Puros) in human maxillary sinus grafts. J Oral Implantol 2005: 31: 171-179.

16. Scarano A, Degidi M, Iezzi G, Pecora G, Piattelli M, Orsini G, Caputi S, Perrotti V, Mangano C, Piattelli A. Maxillary sinus augmentation with different biomaterials: a comparative histologic and histomorphometric study in man. Implant Dent 2006: 15: 197-207.

17. Schlegel KA, Schultze-Mosgau S, Wiltfang J, Neukam FW, Rupprecht S, Thorwarth M. Changes of mineralization of free autogenous bone grafts used for sinus floor elevation. Clin Oral Implants Res 2006:17: 673-678.

18. Schwartz Z, Goldstein M, Raviv E, Hirsch A, Ranly DM, Boyan BD. Clinical evaluation of demineralized bone allograft in a hyaluronic acid carrier for sinus lift augmentation in humans: a computed tomography and histomorphometric study. Clin Oral Implants Res 2007: 18: 204-211.

19. Serra E Silva FM, Ricardo de Albergaria-Barbosa J, Mazzonetto R. Clinical evaluation of association of bovine organic osseous matrix and bovine bone morphogenetic protein versus autogenous bone graft in sinus floor augmentation. J Oral Maxillofac Surg 2006: 64: 931-935.

20. Steigmann M, Garg AK. A comparative study of bilateral sinus lifts performed with platelet-rich plasma alone versus alloplastic graft material reconstituted with blood. Implant Dent2005: 14: 261-266.

21. Tadjoedin ES, de Lange GL, Lyaruu DM, Kuiper L, Burger EH. High concentrations of bioactive glass material (BioGran) vs. autogenous bone for sinus floor elevation. Clin Oral Implants Res 2002: 13: 428-436.

22. Thor A, Wannfors K, Sennerby L, Rasmusson L. Reconstruction of the severely resorbed maxilla with autogenous bone, platelet-rich plasma, and implants: 1-year results of a controlled prospective 5-year study. Clin Implant Dent Relat Res2005:7: 209-220.

23. Thor A, Franke-Stenport V, Johansson CB, Rasmusson L. Early bone formation in human bone grafts treated with platelet-rich plasma: preliminary histomorphometric results. Int J Oral Maxillofac Surg2007: 36: 1164-1171.

24. Thorwarth M, Srour S, Felszeghy E, Kessler P, Schultze-Mosgau S, Schlegel KA. Stability of autogenous bone grafts after sinus lift procedures: a comparative study between anterior and posterior aspects of the iliac crest and an intraoral donor site. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005:100: 278-284.

25. Wallace SS, Froum SJ, Cho SC, Elian N, Monteiro D, Kim BS, Tarnow DP. Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: histomorphometric and clinical analyses. Int J Periodontics Restorative Dent 2005: 25: 551-559.

26. Wiltfang J, Schlegel KA, Schultze-Mosgau S, Nkenke E, Zimmermann R, Kessler P. Sinus floor augmentation with beta-tricalciumphosphate (beta-TCP): does platelet-rich plasma promote its osseous integration and degradation? Clin Oral Implants Res 2003: 14: 213-218.

27. Zizelmann C, Schoen R, Metzger MC, Schmelzeisen R, Schramm A, Dott B, Bormann KH, Gellrich NC. Bone formation after sinus augmentation with engineered bone. Clin Oral Implants Res 2007:18: 69-73.

Chapter 3

Comparison between conventional and piezoelectric surgical tools for maxillary sinus floor elevation.

A randomized controlled clinical trial.

This chapter is an edited version of the manuscript.

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Abstract

Aim: To assess the performance of conventional rotative instruments and a piezoelectric device for maxillary sinus floor elevation surgery in a randomized clinical trial.

Materials & Methods: Thirty-six consecutive patients (59.2±10.7 years, range 38-76 years) needing bilateral sinus floor elevation surgery agreed to participate in this study. In a parallel split mouth design randomized clinical trial, in which the allocation of the surgical technique to be used on the determined sites was randomly assigned, one site was always treated with conventional rotative instruments (control group) and the other site with piezosurgery (test group). In addition, in a random order, the grafted sites were covered with a collagen membrane or no membrane. After a healing period of 3-4 months implants were placed.

Results: Comparison of clinical features of the test and control sites revealed no differences with regard to wound healing and complications (perforations of the sinus membrane) during or post surgery ($p=0.458$, $p=1.0$, respectively). A clinically insignificant, but statistically shorter operation time was observed when using conventional rotative instruments (11.1±2.4 min) than using piezosurgery (15.1±2.9 min; $p<0.001$). In both groups, application of a resorbable membrane did not result in less horizontal bone resorption (membrane: 1.43 mm, no membrane: 1.06 mm; $p=0.062$); All 193 implants could be placed with primary stability. One year after functional loading survival rate was 100%.

Conclusion: It can be concluded that for maxillary sinus floor elevation surgery piezoelectric device forms a reliable alternative to the use of conventional rotative instruments.

Introduction

The application of implant based prosthodontics has evolved into a viable alternative to conventional prosthetic procedures. However, implant procedures in the posterior maxilla often pose a problem due to an insufficient pre-existent bone volume (1). This restriction is not reserved to edentulous patients, but is also often observed in partially dentate patients needing an implant-based prosthodontic reconstruction in this region. An insufficient volume of bone to allow for reliable primary placement of implants can be solved by a maxillary sinus floor elevation procedure using autogenous bone and/or bone substitutes (2, 3). Such approaches are in need of access to the maxillary sinus.

Many surgical techniques have been used to get access to the maxillary sinus via the lateral wall allowing for elevation of the sinus membrane. The most common intraoperative complication of the various surgical approaches is perforation of the Schneiderian membrane, with perforation rates of 7% up to 56% reported in the literature (4, 5). In most instances, perforation occurs either while using rotative instruments to make the window or when using hand instruments to gain initial access to begin the elevation of the membrane from the sinus walls.

More recently, in line with the tendency towards minimally invasive surgery, the use of ultrasonic waves for bone cutting has been introduced in oral and maxillofacial surgery. An important achievement of this approach, using a piezoelectric device, is the much lower risk on causing visible injury to the adjacent soft tissues. The piezoelectric device has been reported to decrease the risk of damage to surrounding soft tissues and many other critical structures (nerves, vessels) (6-8). Wallace and colleagues (8) have shown in a series of 100 consecutive cases using the piezoelectric technique, that when using a piezoelectric device in stead of rotative instrumentation the risk of perforations of the Schneiderian membrane dropped from 30% to 7%. Furthermore, all perforations with the piezoelectric technique occurred in their study during the hand instrumentation phase and not with the piezoelectric inserts. However, their study is limited because it lacked a control group. In the current study we tested the hypothesis that a piezoelectric device during maxillary sinus floor elevation, was non inferior to conventional rotative instruments with respect to bone healing, operation time, and complications per and post surgery. Additionally we tested whether application of a resorbable membrane reduces resorption of an augmented site.

Material en Methods

Patients

Thirty six consecutive patients (age 59.2±10.7 years, range 38-76 years, 21 female, 15 male) fulfilling the inclusion criteria mentioned below agreed to participate in this study. The patients had been referred to the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen because of insufficient retention of their upper denture related to a severely resorbed maxilla. The patients were edentulous in the maxilla. Patients were selected using the following inclusion criteria:

- severely resorbed maxilla (class V-VI, Cawood and Howell (9)) with reduced stability and retention of the upper denture;
- edentulous period of at least one year;
- no history of radiotherapy in the head and neck region;
- no history of reconstructive pre-prosthetic surgery or previous implant surgery;
- no pathology in maxillary sinus.

In all patients maxillary overdentures were planned supported by 4 to 6 implants. Informed written consent to participate in this study was obtained from all patients.

Orthopantomograms, lateral cephalometric analysis, and postero-anterior oblique radiographs were made to assess the height of the maxillary alveolar bone, the dimensions of the maxillary sinus, and the anterior-posterior relationship of the maxilla to the mandible. The radiographs were also screened for sinus pathology. The mean vertical height of the alveolar bone on the orthopantomogram between the top of the alveolar crest and the sinus floor was 3±2 mm (range 1-5 mm).

Study design

All 36 patients were treated with a bilateral sinus floor elevation procedure with conventional rotative instruments and piezosurgery. Randomly, by envelopes, on one side the elevation procedure was performed with conventional rotative instruments and on the other side with piezoelectric bone surgery (Piezosurgery, Mectron Medical Technology Spa, Carasco, Genoa, Italy) (6, 7, 10). Furthermore, to assess whether there was a need to apply a resorbable collagen membrane to reduce bone resorption in a horizontal direction related to either the conventional or piezo approach, in a random order either or not a resorbable membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the grafted area.

Per patient the following variables were analyzed:

- operation time;
- width of alveolar crest, after mucoperiostal flap, measured with a caliper on six points per jaw (3 right, 3 left) before and 3-4 months after sinus floor elevation;
- application of a resorbable membrane (at random);
- complications per-/post surgery;
- dehiscences.

Surgical protocol

The maxilla of the patients was reconstructed with autogenous anterior iliac crest bone grafts under general anaesthesia. In all cases, bilaterally a two-stage procedure (first stage: bone grafting; second stage: placement of implants) had to be performed because the height of the maxillary bone and/or the width of the alveolar crest were less than 5 mm. A bone height of 5 mm or more is thought to be prerequisite for implant placement with sufficient primary stability (11, 12). In addition to elevation of the floor of the maxillary sinus the width of the alveolar crest was reconstructed.

All the surgeries were carried out by two surgeons (one harvesting the iliac crest bone graft, one performing the sinus elevation surgery). Using the surgical procedure described by Raghoebar and colleagues (11), an osteotomy was made in the lateral wall of the maxillary sinus after a pedicled mucoperiostal flap was raised to expose the lateral wall of the maxillary sinus with or a conventional rotative bur or piezosurgery. All bone grafts were harvested from the anterior iliac crest. Subsequently, the monocorticocancellous iliac crest bone grafts were placed buccally of the cortex of the alveolar defect in order to increase the width of the superior alveolar process. The "remaining" graft was ground in a bone mill (Stryker Leibinger, Freiburg, Germany). The cancellous side of the bone graft was in contact with the maxillary bone and again cancellous bone particles were used to fill the small gaps between the bone graft and the alveolar crest. The bone blocks were fixed to the alveolar bone with 6 titanium screws (Martin Medizin Technik, Germany) (diameter 1.5 mm, length 10 mm). After the bone blocks were placed, the horizontally bone width was measured at the spot of the screws with a calliper to the nearest half of a millimeter (pre-augmentation width) as described by von Arx & Buser (13). Per patient six measurements per jaw (three on each site) were done. Randomly, per envelopes, at one treated site, a collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the facial sinus wall defect on the surface of grafted sites, the other side was left uncovered. The mucoperiostal flap was replaced and wound closure was performed by using resorbable suture material Vicryl 4.0 (Ethicon, Norderstedt, Germany).

Before harvesting the bone grafts, the patients received broad-spectrum antibiotics, starting one hour preoperatively (intravenously) and continued orally for 2 days after surgery. Postoperatively, the patients received an aqueous 0.2% chlorhexidine mouth rinse (1 minute, 3 times daily) for 2 weeks. One month postoperatively, the edentulous patients were allowed to wear their dentures, after relining them in the operated areas with a soft liner.

After a healing period of 3-4 months, second stage surgery was done under general anaesthesia in the day clinic. After reflecting the mucoperiostal flap, the width of the reconstructed alveolar crest was measured again at the spot of the screws with a calliper.

Thereafter the titanium screws were removed and implants were inserted. In all cases the bone volume was sufficient and a total of 193 nonsubmerged one-piece implants (Straumann (ITI)®, Dental Implant System, Institut Straumann, Waldenburg, Switzerland) with

adequate primary stability could be placed. Three months after insertion the prosthetic construction was fabricated.

Clinically, all patients were evaluated according to a standardized protocol 1, 3, 6 and 12 weeks after surgery by a clinical research not knowing which procedure had been performed at a particular site. The clinical protocol included assessment of complications during surgery and postoperative healing (inflammation, redness of the mucosa, wound dehiscence, sequestration, and loss of bone particles). Furthermore, patients were followed up to one year after functional loading.

For statistical analysis a t-test and for analysis of time a linear regression analysis were used. All 36 patients were included for analysis. A p-value of <0.05 was considered as a significant result.

Results

Clinical results

In all 36 patients healing was uneventful and all patients could be supplied with an adequately implant placement and functioning implant-supported maxillary overdenture. In all cases there was adequate bone and all 193 implants were placed with primary stability. Loss of bone particles through the nose was not observed.

Surgery

Operation time was significantly shorter when using conventional rotative instruments (11.1±2.4 min) than when using piezosurgery (15.1±2.9 min), ($p<0.001$; linear regression analysis).

Width of the alveolar bone

In all cases bone healing was uneventful and no problems were seen. Therapy (conventional rotative bur versus piezosurgery) had not significantly influenced horizontal bone widththree months after sinus floor elevation. The average bone (mean±sd) width at the beginning was 7.5±0.2 mm in the conventional treated group and 7.6±0.4 mm in the piezogroup. Three to four months after sinus lift surgery the bone the average bone width had reduced to 6.2±0.2 mm in the conventional group and 6.3±0.3 mm in the test group ($p=0.523$, t-test). All measurements were performed in the 3-4 month post sinus augmentation surgery period (14.6 ±2.6 weeks; range 12-17 weeks).

Application of a resorbable membrane

Application of a resorbable membrane had not reduced horizontal bone resorption three months after sinus floor elevation, both within and between groups (p=0.062; t-test). During the healing period, the width of the sites covered with a membrane reduced from 7.4±0.3 mm to 6.0±0.2mm and at the sites not covered with a membrane from 7.6±0.2 mm to 6.6±0.3 mm.

Perforation of the sinus membrane

In total, 8 sinus membrane perforations occurred, four in each group (p=1.0; t-test).

Dehiscences after implantation procedure

In total 8 dehiscences (in 8 patients) occurred during implant placement on the buccal side. All dehiscences were covered with autogenous bone and bovine bone mineral (BioOss®, Geistlich Biomaterials, Wolhusen, Switzerland). A collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the defects. Wound healing was uneventful. The occurrence of a dehiscence of the implant was not related to the type of surgery applied during the sinus floor elevation surgery (p= 1.0; t-test).

Implants

In all augmented regions, all 193 implants could be installed with primary stability. On average the implants had been placed 14.6 weeks (range 12-17 weeks) post-augmentation. Healing was uneventful and all patients could be supplied with an adequately implant placement and functioning implant-supported maxillary overdenture. One year implant survival rate (one year after functional loading) was 100%.

Discussion

The present randomized-controlled clinical trial asessed the performance of conventional rotative instruments and a piezoelectric device during maxillary sinus floor elevation, with respect to bone healing, application of a membrane, operation time, and complications per and post surgery. It was shown that piezoelectric bone surgery is a reliable alternative to the use of conventional rotative instruments as the results of both techniques were comparable. This observation is in agreement with the clinical results reported by Barone and collegues (15). The only limitation of piezosurgery observed in this study was the time factor as the operation time was significantly shorter when using conventional rotative instruments. This observation is in agreement with the studies of Kotrikova and colleagues,

Barone and colleagues and Landes and colleagues (14-16), but the difference in operation time between both operative procedures for maxillary bone is, from a clinical perspective, negligible. However, in areas with a higher bone structure or thickness, the extra time needed for making an osteotomy by piezoelectric surgery can be much higher, up to 5-fold and even more (14).

The perforation of the Schneiderian membrane represents the most frequent complication in standard sinus lift surgery using rotative instruments. Torella and colleagues (17) reported a reduced risk of perforating the Schneiderian membrane using normal ultrasound instruments for the opening of the bony window. They posed that inadvertent perforations of the sinus membrane are unlikely when piezosurgical techniques are appropriately applied. In addition, in a series of 21 bony window and membrane elevations performed with piezoelectric surgery, only one perforation was reported, which resulted in a 95% success rate (10). In our study comparison of the clinical features at the test (piezosurgery) and control (conventional rotative instruments) revealed no differences with regard to perforations of the sinus membrane during surgery. Barone and colleagues (15) showed a higher number of membrane perforations noted with piezosurgery than we observed in our trial, but the differences in their study also did not reach the level of significance. Therefore, it may be concluded that risk on sinus membrane perforation is comparable between the use of piezosurgery or a conventional rotative bur, at least from a clinical perspective. Probably, it is the experience of the surgeon in using conventional rotative instruments instead of piezoelectric surgery that is leading whether perforations will occur and what the consequences of such perforations will be. That also means that piezoelectric surgery only is reliable if the surgeon does have sufficient experience in using piezoelectric surgery and reverse for rotative instruments.

In previous reports were no differences found in implant survival with respect to membrane perforations (4, 18). Also our study showed a one year implant survival rate of 100% in both groups.

Application of a resorbable membrane did not significantly reduce post augmentation loss of bone width, which is in agreement with the observations of Gielkens and colleagues (19). The latter authors studied the effect of membrane coverage on resorption and incorporation of autogenous onlay bone grafts in rats. In that study, it was concluded that application of a membrane barrier is not necessary to prevent bone resorption. Furthermore, the present study showed that application of a membrane does not have any significant influence on the operation time in addition applying a membrane increases the costs of treatment.

From this randomized-controlled clinical trial comparing the performance of conventional rotative instruments and a piezoelectric device during maxillary sinus floor elevation, with respect to bone healing, operation time, and complications per-/post surgery, it can be concluded that piezoelectric bone surgery forms a reliable alternative to the use of conventional rotative instruments except to time of surgery. This limiting factor seems not to be clinically relevant for maxillary sinus floor elevation surgery.

References

1. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM et al. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants Res 2004: 15: 724-732.

2. Boyne PJ & James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg 1980: 38: 613-616.

3. Tatum H, Jr. Maxillary and sinus implant reconstructions. Dent Clin North Am 1986: 30: 207-229.

4. Schwartz-Arad D, Herzberg R, Dolev E. The prevalence of surgical complications of the sinus graft procedure and their impact on implant survival. J Periodontol 2004: 75: 511-516.

5. Pjetursson BE, Tan WC, Zwahlen M, Lang NP. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 2008: 35: 216-240.

6. Vercellotti T. Technological characteristics and clinical indications of piezoelectric bone surgery. Minerva Stomatol 2004: 53: 207-214.

7. Eggers G, Klein J, Blank J, Hassfeld S. Piezosurgery: an ultrasound device for cutting bone and its use and limitations in maxillofacial surgery. Br J Oral Maxillofac Surg 2004: 42: 451-453.

8. Wallace SS, Mazor Z, Froum SJ, Cho SC, Tarnow DP. Schneiderian membrane perforation rate during sinus elevation using piezosurgery: clinical results of 100 consecutive cases. Int J Periodontics Restorative Dent 2007: 27: 413-419.

9. Cawood JI & Howell RA. A classification of edentulous jaws. Int J Oral Maxillofac Surg 1988: 17: 232-236.

10. Vercellotti T, De Paoli S, Nevins M. The piezoelectric bony window osteotomy and sinus membrane elevation: introduction of a new technique for simplification of the sinus augmentation procedure. Int J Periodontics Restorative Dent 2001: 21: 561-567.

11. Raghoebar GM, Timmenga NM, Reintsema H, Stegenga B, Vissink A. Maxillary bone grafting for insertion of endosseous implants: results after 12-124 months. Clin Oral Implants Res 2001: 12: 279-286.

12. Chao YL, Chen HH, Mei CC, Tu YK, Lu HK. Meta-regression analysis of the initial bone height for predicting implant survival rates of two sinus elevation procedures. J Clin Periodontol 2010: 37: 456-465.

13. von Arx T, Buser D. Horizontal ridge augmentation using autogenous block grafts and the guided bone regeneration technique with collagen membranes: a clinical study with 42 patients. Clin Oral Implants Res 2006: 17: 359-366.

14. Kotrikova B, Wirtz R, Krempien R, Blank J, Eggers G, Samiotis A, et al. Piezosurgery-a new safe technique in cranial osteoplasty? Int J Oral Maxillofac Surg 2006: 35: 461-465.

15. Barone A, Santini S, Marconcini S, Giacomelli L, Gherlone E, Covani U. Osteotomy and membrane elevation during the maxillary sinus augmentation procedure. A comparative study: piezoelectric device vs. conventional rotative instruments. Clin Oral Implants Res 2008: 19: 511-515.

16. Landes CA, Stubinger S, Rieger J, Williger B, Ha TK, Sader R. Critical evaluation of piezoelectric osteotomy in orthognathic surgery: operative technique, blood loss, time requirement, nerve and vessel integrity. J Oral Maxillofac Surg 2008: 66: 657-674.

17. Torrella F, Pitarch J, Cabanes G, Anitua E. Ultrasonic ostectomy for the surgical approach of the maxillary sinus: a technical note. Int J Oral Maxillofac Implants 1998: 13: 697-700.

18. Ardekian L, Oved-Peleg E, Mactei EE, Peled M. The clinical significance of sinus membrane perforation during augmentation of the maxillary sinus. J Oral Maxillofac Surg 2006: 64: 277-282.

19. Gielkens PF, Schortinghuis J, de Jong JR, Paans AM, Ruben JL, Raghoebar GM, et al. The influence of barrier membranes on autologous bone grafts. J Dent Res 2008: 87:1048-1052.

Chapter 4

Bone marrow concentrate and bovine bone mineral for sinus floor augmentation.

A controlled, randomised, single-blinded clinical and histological trial: Per-Protocol Analysis.

This chapter is an edited version of the manuscript.

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Abstract

Aim: To evaluate the potential of substituting autogenous bone by bone marrow aspirate concentrate. Both were tested in combination with a bovine bone mineral (BioOss®), for their ability of new bone formation in a multi-centric, randomized, controlled, clinical and histological non-inferiority trial.

Material and Methods: 45 severely atrophied maxillary sinus from 26 patients were evaluated in a partial cross-over design. As test arm, 34 sinus of 25 patients were augmented with bovine bone mineral (BioOss®) and bone marrow aspirate concentrate containing mesenchymal stem cells. Eleven control sinus from 11 patients were augmented with a mixture of 70% bovine bone mineral (BioOss®) and 30% autogenous bone. Biopsies were obtained after 3-4 months healing period at time of implant placement and histomorphometrically analysed for new bone formation.

Results: New bone formation was 14.3±1.8% for the control and non-significantly lower at 12.6±1.7% for the test. Values for bovine bone mineral (BioOss®) of 31.3±2.7% were significantly higher for the test compared to control of 19.3±2.5% ($p<0.0001$). Non-mineralized tissue was lower by 3.3% in the test compared to control (57.6%; $p=0.137$).

Conclusions: New bone formation after 3-4 months is equivalent in sinus, augmented with bone marrow aspirate concentrate and bovine bone mineral or a mixture of autogenous bone and bovine bone mineral (BioOss®). This technique could be an alternative to using autografts to stimulate bone formation.

Introduction

During physiological bone healing osteoprogenitor cells migrate into the defect, differentiate into osteoblasts and produce calcified matrix. These osteoprogenitor cells derive from multipotent mesenchymal stem cells (MSCs) that are recruited from bone marrow. Mesenchymal stem cells can bare low oxygen concentrations and have the potential for osteogenic, chondrogenic and adipogenic differentiation, depending on the external stimulus by cytokines (1-6). To stimulate bone healing, various grafting techniques have been developed to supply either cytokines, progenitor cells, suitable scaffolds or any combination of them (7-10). Adding cytokines can increase bone formation dynamics. Adding progenitor cells should optimise the initial physiologic bony regeneration. Scaffolds should ensure volume stability. Our group gained clinical experience with a resorbable biomaterial and expanded osteoblast-like-cells (11). The constructs did not preserve shape and volume of the augmented area. This is why in the presented study a rather stable osteoconductive biomaterial was used.
Maxillary sinus floor elevation and secondary implant placement is a standardized procedure and therefore a good clinical model to evaluate bone regeneration.
It is common to combine autologous bone with alloplastic material (12, 13). Drawback of the procedure is, that in order to obtain autologous bone, an additional surgical step is needed, causing donor side morbidity (14-16). If alloplastic materials are used without addition, normally longer healing times are chosen, to compensate for the initial lack of regeneration. This explains why there are no reports about healing periods of less than 6 months when pure alloplastic material is used. In comparison to bone harvesting the aspiration of autogenous bone marrow is a minimal invasive puncture, causing less discomfort to the patients. There are case reports on the efficiency of bone marrow aspirate derived stem cell transplantation to improve fracture or non-union healing (17-19). The here presented clinical study histomorphometrically evaluates the bone forming potential of bone marrow aspirate concentrate harvested intra-operatively from the posterior iliac crest and concentrated with a closed system (BMAC, Bone Marrow Concentrate Aspirate) after a short healing time (3–4 months) in sinus lifts of severely atrophied maxillae. As control augmentation served a standard mixture of autogenous bone and alloplastic material.

Material and Methods

Protocol specifications

The clinical study was approved by the ethics committees of the Universities of Freiburg (Germany) and Mainz (Germany). First the ethics committee regarded treating patients with highly resorbed maxillae (2-3mm bone height) and a short, previously unreported, healing time of 3-4 months with only bovine bone mineral (BBM) in the view of a superiority design as unethical. This is why it was decided that the control group should be bovine bone mineral and autogenous bone resulting in a non-superiority design.

The study was conducted in accordance with the Declaration of Helsinki (Fifth revision, 2004). Patients older than 18 years with need of dental implant placement in the posterior maxilla were eligible if they had a maximum of 4 mm residual alveolar height. They had to be able to comply with study related procedures, such as returning for follow-up examinations, exercising good oral hygiene, and being able to understand the nature of the proposed surgery. Written informed consent was obtained prior to any study related procedures. Exclusion criteria were smoking, history of malignancy, radiotherapy or chemotherapy, pregnancy or nursing, general contraindications for dental or surgical treatment; medications, treatments or diseases, which may have an effect on bone remodelling, bone or connective tissue metabolism, or an allergy to collagen.
Primary parameter of the study was new bone formation. Secondary parameters were volume of the augmentate and bone height.

Patient population

133 Patients were assessed for the study. 40 Patients with 70 atrophied sinus were eligible. As the study was designed to evaluate early new bone formation, 14 patients (25 sinus) were excluded due to protocol violations, that would have lead to new bone formation-bias. The main reason was that the implantation was outside the 3-4 months healing time. The patients remaining included 45 severely atrophied sinus from 26 patients (age 56.6±8.0 years; range: 38.9-67.7 years). These patients were treated according to the protocol and were included in the per-protocol evaluation.
Each sinus was randomly assigned to either control or test-arm. Randomisation envelopes in blocks of 6 were generated in a 1:2 ratio for test and control. In the test arm, 34 sinus (25 patients) were augmented with bovine bone mineral (Geistlich Bio-Oss®, Geistlich Biomaterials, Wolhusen, Switzerland) and bone marrow aspirate concentrate (BMAC). 11 control sinus (11 patients) were augmented with a mixture of 70% bovine bone mineral (Bio-Oss®) and 30% autogenous bone harvested from the retromolar area. In 10 patients the randomization resulted in a split-mouth-model in which one side was treated as test arm while the contra-lateral side was control. Due to the surgical intervention neither surgeon, nor patient could be effectively blinded.

Harvesting of Bone marrow aspirate

The pelvic bone was punctured 2 cm latero-caudally from the superior posterior iliac spine. In a 60 ml syringe, flushed with heparin solution (Sodium-Heparine, 10.000 U/ml, diluted with NaCl to 1000 U/ml, both B. Braun, Melsungen, Germany) and then filled with 8ml of citric acid (BMAC-Kit, Harvest Technologies Corporation, Plymouth, MA, USA), 52ml of bone marrow were collected. According to the instructions of the manufacturer bone marrow cells were isolated in 15 minutes directly in the operating room by using the BMAC system (Bone Marrow Procedure Pack, Harvest Technologies Corporation, Plymouth, MA, USA).

Colony Forming Unit Assay

To evaluate the number of MSCs a probate amount of aspirate was used for cell count (Sysmex, Norderstedt, Germany) and continuous growth from selected patients. Cells were plated on 96-well plastic plates (Becton Dickinson, Los Angeles, CA) and cultured in MSCBM-medium (Cambrex, USA) in a humidified atmosphere of 95% air, 5% CO_2 at 37°C for 7 days. Each sample was plated at a density of 10,000 nucleated cells per cm^2. The medium was initially changed after 24 hours and then every second day. Non-adherent cells were washed from the culture. MSCs were selected on the basis of plastic-adhesion. Colonies were counted under an inverted phase-contrast microscope at a final magnification 50x.

Sinus Augmentation Procedure

Under general anaesthesia or local anaesthesia a mucoperiostal flap was raised to expose the lateral wall of the maxillary sinus. A bone window of 1.5cm^2 was outlined with a round burr at 800 rpm with constant saline irrigation. Then the Schneiderian membrane was detached from the sinus floor and lifted. Test sites were augmented with a combination of bovine bone mineral (Bio-Oss® 0.25-1mm, Geistlich Pharma AG, Wolhusen, Switzerland) and bone marrow aspirate concentrate with autologous thrombin made from venous blood (Thrombin Kit, Harvest Technologies Corporation, Plymouth, MA, USA). The thrombin was needed to clot the BMAC-solution around the bovine bone mineral. 3ml of bone marrow concentrate and 1ml of autologeous thrombine solution were added with a two-chamber-syringe to 2g of biomaterial with a volume of 4cm^3 (20). The biomaterial was applied according to clinical needs.
Control sites were augmented with a mixture of bovine bone mineral (Bio-Oss®) (70%) and milled autogenous bone (30%). No blood was added to the mixture. Autogenous bone was obtained from the retromolar area as described by Sauvigne et al. (21).
A collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was placed over the facial sinus wall to cover the graft. The mucoperiostal flap was replaced and closed with resorbable suture material (Vicryl 4-0; Ethicon, Norderstedt, Germany). Patients were discharged from the hospital the same day. After an average healing period of 3.41±0.39 months second stage implant placement was performed under local anaesthesia. Cylindrical bone biopsies from the augmented maxillary sinus were taken with a trephine burr (Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) under 600 r.p.m. and constant cooling.

Volume Rendering

For 29 test sinus and 9 control sinus cone beam digital volume tomography (dental CT) was recorded due to clinical needs (Scanora, Soredex, Schutterwald, Germany and Promax, Planmeca, Bielefeld, Germany). After each 2mm-slice was outlined manually the vol-

ume of the augmentation at the time of implant insertion was calculated by the program VoXim 4.3 (© IVS Solutions AG, Germany).

Histological Evaluation

Bone biopsies were fixed in formalin for 48 hours, rinsed with water and dehydrated in serial steps of alcohol (70%, 80%, 90% and 100%) remaining for 3 days in each concentration. Then samples were infiltrated with resin for 2 weeks (Technovit 7200 VLC, Heareus Kulzer, Hanau, Germany). The resin was polymerized in a UV light chamber for 10 hours. After hardening of the polymer, two sections of 300–400µm thickness were cut parallel to the trephine axis (Microslice, IBS, Cambridge, GB). Sections were placed on acrylic slides (Maertin, Freiburg, Germany) and reduced to a thickness of 100µm on a rotating grinding plate (Struers, Ballerup, Denmark). Specimens were stained with Azur II and Pararosanilin. Histologic examination was performed with a light microscope (Axiovert 135, Zeiss, Kochern, Germany). Pictures were stored digitally. Bovine bone mineral, autogenous bone and new bone formation were marked manually on the screen (biomaterial=green, old bone=yellow, newly formed bone=red) while the actual specimen could be viewed under the microscope. Due to the short healing time the local bone was clearly distinguishable from the augmentation site. Histomorphometric analysis was achieved by detection of RGB-colours with the computer software AnalySISD Soft Imaging system (Olympus Europa GmbH, Hamburg, Germany). Only the augmentation site was evaluated for the histomorphometrical analysis and was marked as region of interest (ROI) before. The differentiation between bovine bone mineral particles, autogenous bone and new bone formation-tissue was possible because osteocytes can be detected in the lacunae of new bone formation and autogenous bone. New bone formation was stained darker than autogenous bone and shows lamellar bone formation. The overall evaluated area was divided in bovine bone mineral, newly formed bone, old bone, and non-mineralised tissue.

Proof of Multipotency

MSCs obtained from the bone marrow aspirate concentrate were amplified and differentiated into three cell lineages according to Pittinger et al. (22) The lines were tested for their multipotent stem cell character in their ability to differentiate into chondrogenic, adipogenic and osteogenic phenotypes. Adipocytes were stained with oil red O, a lipophilic red dye. Chrondrogenic potential was confirmed by immunostaining with mouse anti-human aggrecan antibodies for aggrecan (counterstaining DAPI, Sigma, St. Louis, MO, USA) and collagen type II (both from Sigma, St. Louis, MO, USA). Osteogenic cells were tested for their expression of high levels of alkaline phosphatase (counterstaining with neutral red, both from Sigma, St. Louis, MO, USA), collagen type I (counterstaining with Mayer's Hemalum solution, Merck, Darmstadt, Germany) and calcification was assessed with the van Kossa staining.

Statistical Analysis

The study is a pilot study, as no data could be identified to give a thorough base for a power analysis. For the parameters New Bone (new bone formation) and bovine bone mineral values were expressed in percentage of the evaluated area.
Data were analysed by a linear mixed effect model with power weighting to correct for unequal variances (23). Error bars giving standard errors and p-values are based on tests of linear contrasts. This model correctly handles correlation in the partial cross-over design used in this study.

Results

26 Of 40 randomized patients (45 sinus) were treated according to the protocol including all visits in the set time window and were included in the per-protocol-analysis. Patients were treated between May 2006 and March 2008. The most prevalent reason for patient drop-out/removal from evaluation was late implant placement outside the 3-4-months-interval given for the healing time. Removal of these patients from the analysis was necessary to evaluate early bone formation, the aim of the study.
All patients from the test arm (Bio-Oss® + bone marrow aspirate concentrate) recovered well from the surgical procedure. No major intraoperative complications occurred. Occasional rupture of the sinus membrane was treated by placing a membrane (Bio-Gide, Geistlich Pharma AG, Wolhusen, Switzerland) over the rupture. No occurrence of pain, haematoma or infection at any time after bone marrow aspiration and sinus floor augmentation in the post operative time were recorded. In the control group (Bio-Oss®+autogenous bone) one patient suffered from an injury of the inferior alveolar nerve, which resulted from the harvesting of the retromolar bone. Two patients of this group had an infection of the bone harvesting site. All noticed complications healed within reasonable time and standard care.

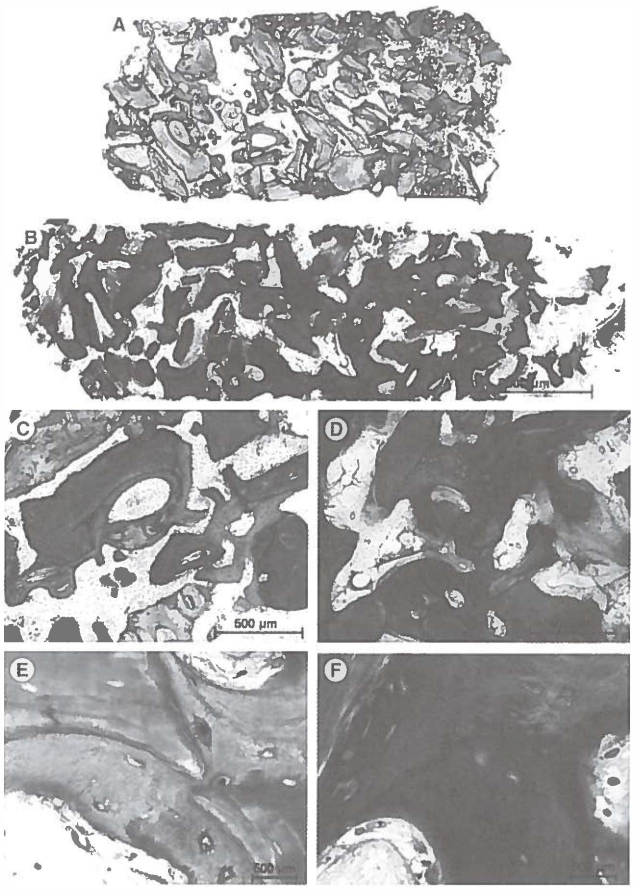
Healing Time

Average healing time was 3.46±0.43 months for the test group treated with bone marrow aspirate concentrate and Bio-Oss® and with 3.34±0.42 months very similar for the control group treated with autogenous bone in combination with Bio-Oss®.

Volumetric analysis

For 29 test samples and 9 control samples the radiographic bone volume was determined. Radiologic gain and persistence of augmented bone height, was statistically higher for the test (1.74±0.69ml) than for the control group (1.33±0.62ml) (p = 0.02; 95%-confidence interval of the difference 0.13 to 1.04ml).

Figure 1 Histological analysis of both test and control specimens. Overview of a whole obtained specimen: (A) control, (B) test site. (C, D) Control and test sites at x5 magnification. (C) The network of newly formed bone around the autogenous bone and bovine bone mineral (BBM) particles. The autogenous bone is in the process of being resorbed and replaced by newly formed bone. (D) A similar network of newly formed bone around BBM particles. No indication of BBM resorbtion can be seen. (E, F) Control and test site at x40 magnification. Both show areas of woven bone and indication of early remodeling into lamellar bone.



Histological analysis

Specimens obtained from both the test and the control sites were histologically similar (Figure 1). No signs of inflammation were detected. Vital bone tissue containing osteocytes inside the bone lacunae was observed in the newly formed osseous lamellae. The biomaterial could be easily identified by its size, shape and colour in comparison to new bone fromation or the pre-existing local bone. New bone formation appeared darker than bovine bone mineral particles in the Azur II Pararosanilin staining (Figure 1). The newly formed bone connected the biomaterial particles and stabilized the grafted complex. New bone formation was also observed in the macro-pores of the Bio-Oss® particles. Blood vessels could be detected throughout the specimens showing that the blood supply is ensured throughout the whole augmentate. Generally the biomaterial with the new bone formation was well integrated in the surrounding local bone (Figure 1). The maturity of the newly bone formation was assessed visually. All specimens showed mainly woven bone, with regions of bone maturation and partially lamellar structure (Figure 1).

Histomorphometrical analysis

An overview of the estimated values and the 90%-confidence-intervals of new bone formation, biomaterial and marrow space are displayed in Figure 2.
New bone formation: Average of new bone formation was 14.3±1.8% in the control group and 12.6±1.7% in the test group. The difference is not statistically significant (p=0.333) with a 90%-confidence-interval for the difference of -4.6% to 1.2% for specimen obtained from test sites, giving a 95%-non-inferiority limit of not more than 4.6 % for bone formation of the test treatment with respect to the control treatment.
Biomaterial: The measured fraction of bovine bone mineral (Bio-Oss®) was significantly higher for the test arm (31.3±2.7%) compared to control (19.3±2.5%; p<0.0001); the difference resembles the mixing ratios of test (100% Bio-Oss®) and control group (70% Bio-Oss®).
Marrow space: The remaining MS of the augmentate 57.7±2.3% in the control arm, was with 3.3% non-significantly lower than the test arm (54.4±2.2; p=0.137).

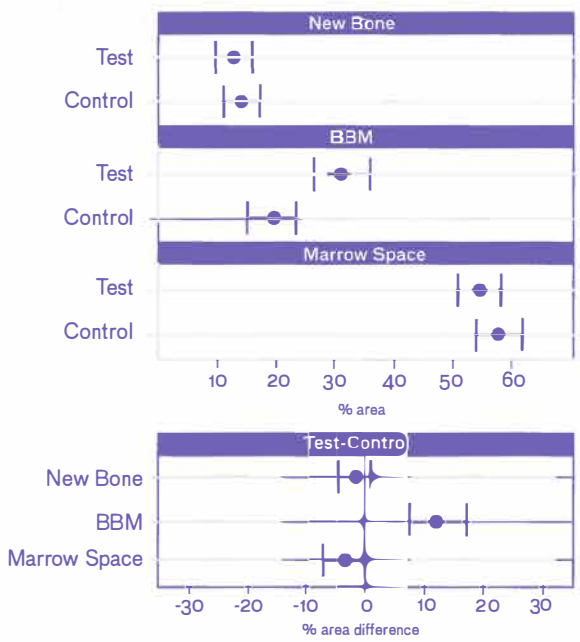
Colony Forming Unit Assay (CFU)

The with 8 ml citric acid anticoagulated aspirate contained $17.2x10^3 \pm 13.5x10^3$ white blood cells(WBC)/µl. The BMAC-process resulted in $79.4x10^3 \pm 45.8x10^3$WBC/µl with 41.4±15.6 CFUs/$1x10^6$ mononuclear cells.

Proof of Multipotency

The cells taken from the BMAC procedure, could be subsequently selected as plastic adherend cells and differentiated successfully into adipocytes as shown by oil red O staining,

Figure 2 Graphic presentation of the histomorphometric analysis. Shown are the mean values with the standard deviation for new bone, BBM, and marrow space. The lower part of the graph shows the comparison of the test and control groups with 90% confidence intervals.



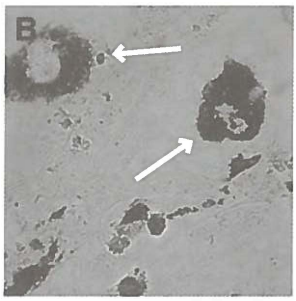
chondrocytes as shown by aggrecan immuno staining and collagene type II activity and osteoblasts as shown by calcification, alkaline phosphatase and collagene Type I activity. This demonstrates their multipotency and proves them to be MSCs (Figure 3).

Discussion

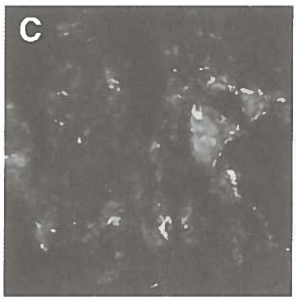
The regeneration of bone requires an adequate scaffold, as well as cells, that are capable to attach to the scaffold, to proliferate and differentiate. The dynamics of bone healing are controlled by growth factors, such as bone morphogenic proteins. To promote these requirements several strategies for bone and tissue regeneration have been developed. However, in the case of sinus floor elevation, the most frequently applied technique is the augmentation with alloplastic biomaterials, such as β-TCP or hydroxyapatite. In addition to being a common procedure, sinus floor elevation presents a good model to any clinical

Figure 3 Test for multipotency of the colony-forming units obtained from the bone marrow aspirate concentrate procedure. Phenotype and staining used:

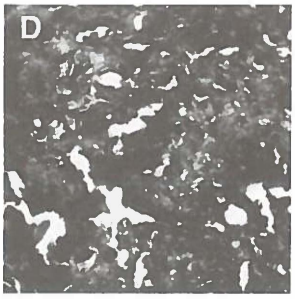
(A) mesenchymal stem cells from colony-forming units: HE staining;



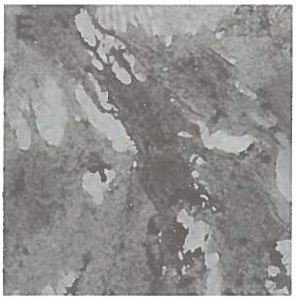
(B) adipocytes: oil red O staining; arrows indicate intracellular fat droplets;



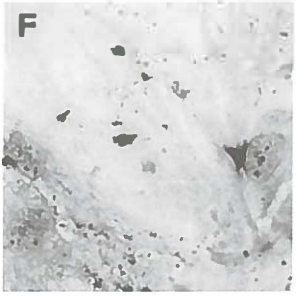
(C) chondrocytes: aggrecan immunostaining;



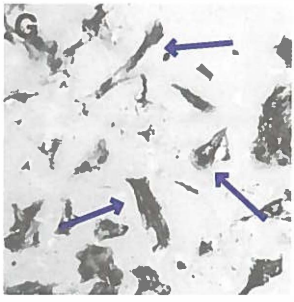
(D) chondrocytes: collagen type II (brown staining is positive, counterstaining with Mayer's Hemalum solution);



(E) osteoblasts: blue staining is positive for alkaline phosphatase;



(F) osteoblasts: collagene type I activity (reddish-brown staining);



(G) calcification by osteoblasts: van Kossa staining. Dark brown staining indicates calcified tissue (arrows).

evaluation of augmentation materials, as bone formation occurs in an enclosed space, in which it can occur with a minimum of external factors. Furthermore, a two step procedure, with delayed implant placement, can provide researchers with bone biopsies, to monitor the bone development at a given moment.

Consequently, many bone substitutes have been evaluated in sinus floor procedures. Bovine bone mineral (Bio-Oss®) is one of the most widely used scaffolds employed in sinus augmentation procedures (24-26). It has similar physical properties to human cancellous bone, both in its morphological structure and its mineral composition (27). Scientific studies have shown that bovine bone mineral only remodelled to a minimal amount, and therefore ensures the augmentation volume (28). The higher bovine bone mineral content could be responsible for the higher volume in the test-arm. This result is in accordance with the animal trial by Gutwald et al. in which sinus augmentations with autogenous bone showed less volume than augmentations with bovine bone mineral (Bio-Oss®) and MSCs (20). Often autologous bone chips, providing preosteoblasts as well as autologous growth factors, are added to increase the kinetics of the initial bone formation. As a result autogenous bone is still the most widely applied augmentation material (12).

This combination of autogenous bone and Bio-Oss® has been investigated in previous histological sinus floor elevation studies. Yildirim et al. first evaluated the bone formation in sinus floor elevations performed with a mixture of autogenous bone and Bio-Oss® in 13 sinus floor elevations and found 18.9±6.4% new bone formation after a 7.1 months healing period (29). In a second study Yildirim et al. examined under similar conditions, sinus floor elevation procedures with only Bio-Oss® and venous blood in 15 sinus of 11 patients and found 14.7% (±5.0%) new bone formation after a healing time of 6.8 months (30). Therefore, the addition of autogenous bone to the Bio-Oss® yielded an advantage of 4.2% new bone formation, which can be translated in a delay in the healing time of a couple of months. Similarly, Thorwarth et al. wanted to further quantify this effect in the bone formation dynamics (31). They have used a mini-pig model, showing that in the first 8 weeks Bio-Oss® with 25% autogenous bone added has superior bone forming kinetics compared to Bio-Oss® alone. For longer healing times this differences in formed bone quantity narrow, and were no longer detectable after 3 months. Arguably, this data cannot be translated one to one from mini-pigs to man, but should be regarded as a guideline that the initial bone formation is significantly higher, if autogenous bone is added. In a previous animal trial Sauerbier et al. found 49 % more bone formation per time when bone marrow derived MSCs were attached to Bio-Oss® with fibrin glue (32). Bovine bone mineral and fibrin glue without cells served as control. This means that the clot alone was not responsible for the improved new bone formation.

Several Tissue Engineering approaches have been tried to stimulate bone formation. Similarly to the results presented here, the addition of in vitro cultured MSCs has proven to stimulate osteogenesis (33, 34). Preclinical and clinical studies have demonstrated the ability of bone marrow derived stem and progenitor cells to regenerate various tissues, including bone (35). Shayesteh et al. presented clinical and histological findings in 6 patients

that suggest that the addition of in vitro cultivated MSCs to hydroxyapatite/β-TCP may enhance the bone formation and allow implant placement after sinus floor augmentation (36). Ueda et al. evaluated the use of tissue-engineered bone with in vitro cultured MSCs and subsequently differentiated osteoblasts, platelet-rich plasma, and β-TCP as grafting materials for sinus lift procedures, and simultaneous implant placement in a study with 20 implants in 6 patients (37). A mean increase of 7.3 ± 4.6 mm in bone height was seen in radiographic findings 12 months after the surgery. The same research group used tissue engineered bone in 14 patients for sinus lift and onlay grafts. This in vitro cultured bone induced bone formation and osseointegration of the placed dental implants (38). To bypass the problem of selection, multiplication and differentiation most of the mentioned studies selected MSCs, multiplied them, and then differentiated them to the osteogenic lineage. As this is unreasonable to do in daily practice, an approach was chosen in which bone marrow aspirate or its concentrate is used unselected and undifferentiated, and therefore. Based on the hypothesis that the number of MSCs is secondary, but their individual potential of MSCs to survive high stress and chemotactically attract osteogenic progenitor cells will dominate. As the augmentation material within the sinus is far from local blood supply, it can be assumed that a natural selection procedure will be applied to the cells. As blood and oxygen supply is re-established, the MSCs could then unfold their full potential (39). In clinical settings it is ethically difficult to prove survival of transplanted cells. Smiler et al. described a small number of cases where bone marrow aspirate from the iliac was placed onto biocompatible scaffolds. The procedure successfully regenerated bone in sinus augmentations and particulated onlay grafts of the maxilla (40).

The presented data, did not show a correlation between the number of obtained MSCs from each BMAC-procedure to the amount of bone formation seen in the histologies (19). The correlation, seen by others is probably masked by the variability of the potential of the MSCs among the patients (41). The BMAC-procedure resulted in a 4.6 fold increase of white blood cells (WBC) from $17.2x10^3 \pm 13.5x10^3/\mu l$ to $79.4x10^3 \pm 45.8x10^3/\mu l$ with 41.4±15.6 CFUs/$1x10^6$ mononuclear cells. This means estimated 2465.7 colony-forming-cells/augmented 1 cm^3.

From the triangle of bone formation, made up of matrix, cells and growth factors, only the growth factors can increase the overall kinetics of bone formation, as seen in the study by Jung et al. (42). The addition of autogenous cells, can only overcome the initial lack phase leading to a higher early bone formation when compared to augmentations performed with bone substitutes. In the presented study the bone marrow aspirate concentrate biomaterial mixture showed new bone formation comparable to the autologous bone biomaterial composition. Bone marrow aspirate concentrate could therefore compensate partially for the normally added autogenous bone, and eliminate the lack phase seen by augmentations with only bone substitutes.

Consequently, the aim of the presented autologous bone marrow aspirate concentrate approach is to achieve a bone formation rate comparable to Bio-Oss® with autologous bone. To test this hypothesis the study was designed to measure the initial bone formation kinetics

and not primarily the maximal bone formation. Therefore, and early time point after a healing phase of only 3–4 months was chosen.

New bone formation over both groups was very similar. The biopsies of the control group showed 14.3±1.8% NBF, those of the treatment group with Bio-Oss® and bone marrow aspirate concentrate resulted in 12.6±1.7% new bone formation. Statistically the newly formed bone of the test group was equivalent to that of the control group which indicates that the new bone formation of the bone marrow aspirate/ Bio-Oss® mixture is equivalent to the that of autogenous bone/ Bio-Oss® after 3–4 months, indicating good early bone formation. Bone formation after 14.8±0.7 weeks was with 17.7±7.3 % even more in the bone marrow aspirate-biomaterial-side than the 12.0±6.6 % in the bone-biomaterial-side in a split-mouth-trial of Rickert et al. on 11 patients (p=0.026) (43). The reason for the higher new bone formation in the test group in the study from Rickert et al. is not completely clear. There the patients were even older (60.8±5.9 years) than in the presented study (56.6±8.0 years). The autogenous bone particles of the control group were smaller in Rickert's study than in the presented multi center study in which 6 different surgeons treated 70 sinus. In the Rickert study 1 surgeon did the complete surgery on 22 evaluated sinus.

The presented study supports the data of Minamide et al. who found in a histomorphometric rabbit study no differences between the spinal fusion treatment using autologous bone mixed with hydroxyapatite and bone marrow derived MSCs and hydroxyapatite (41). Presently, augmentations performed with bovine bone mineral in a blood clot are left for a longer healing time, compared to augmentations preformed with autogenous bone (29, 30). As the bone formation is equivalent in both groups the here presented option of adding bone marrow aspirate concentrate to a biomaterial reduces the healing time or osseointegration time of the implant, and therefore leads to a reduced treatment time and earlier dental rehabilitation of the patient when compared to BBM alone (29, 30).

Conclusion

Bone marrow aspirate concentrate associated to bovine bone mineral (Bio-Oss®) can regenerate an equivalent amount of new bone when compared to autogenous bone mixed with bovine bone mineral (Bio-Oss®) even after a short healing time of 3–4 months. The bone marrow aspirate concentrate mixture seems to fully compensate the benefit known for autogenous bone in early bone formation. Therefore, bone marrow aspirate+ bovine bone mineral (Bio-Oss®) is a new treatment option almost as convenient as the treatment with only biomaterials and as potent as with autogenous bone.

References

1. Ohgushi H, Dohi Y, Tamai S, Tabata S. Osteogenic differentiation of marrow stromalstem cells in porous hydroxyapatite ceramics. J Biomed Mater Res 1993: 27:1401-1407.

2. Ohgushi H, Dohi Y, Katuda T, Tamai S, Tabata S, Suwa Y. In vitro bone formation by rat marrow cell culture. J Biomed Mater Res 1996: 32:333-340.

3. Ohgushi H, Dohi Y, Yoshikawa T, Tamai S, Tabata S, Okunaga K, Shibuya T.J Osteogenic differentiation of cultured marrow stromal stem cells on the surface of bioactive glass ceramics. Biomed Mater Res 1996: 32: 341-348.

4. Okumura M, Ohgushi H, Dohi Y, Katuda T, Tamai S, Koerten HK, Tabata S. Osteoblastic phenotype expression on the surface of hydroxyapatite ceramics. J Biomed Mater Res 1997: 37:122-129.

5. Curran JM, Chen R, Hunt JA. The guidance of human mesenchymal stem cell differentiation in vitro by controlled modifications to the cell substrate. Biomaterials 2006: 27: 4783-4793.

6. Richardson SM, Curran JM, Chen R, Vaughan-Thomas A, Hunt JA, Freemont AJ, Hoyland JA. The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. Biomaterials 2006: 27: 4069-4078.

7. Gautschi OP, Frey SP, Zellweger R. Bone morphogenetic proteins in clinical applications. ANZ J Surg 2007: 77:626-631.

8. Jones AL, Bucholz RW, Bosse MJ, Mirza SK, Lyon TR, Webb LX, Pollak AN, Golden JD, Valentin-Opran A. Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. J Bone Joint Surg Am 2006: 88:1431-1441.

9. Khan SN, Lane JM. The use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in orthopaedic applications.Expert Opin Biol Ther 2004: 4:741-748.

10. Jensen SS, Broggini N, Hjørting-Hansen E, Schenk R, Buser D. Bone healing and graft resorption of autograft, anorganic bovine bone and beta-tricalcium phosphate. A histologic and histomorphometric study in the mandibles of minipigs. Clin Oral Implants Res 2006: 17: 237-243.

11. Voss P, Sauerbier S, Wiedmann-Al-Ahmad M, Zizelmann C, Stricker A, Schmelzeisen R, Gutwald R. Bone regeneration in sinus lifts: comparing tissue-engineered bone and iliac bone. Br J Oral Maxillofac Surg 2010:48: 121-126.

12. Pjetursson BE, Tan WC, Zwahlen M, Lang NP. A systematic review of the success of sinus floor elevation and survival of implants inserted in combination with sinus floor elevation. J Clin Periodontol 2008: 35: 216-240.

13. Nkenke E, Stelzle F. Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clin Oral Implants Res 2009: 20: 124-133.

14. Kalk WW, Raghoebar GM, Jansma J, Boering G. Morbidity from iliac crest bone harvesting. J Oral Maxillofac Surg 1996: 54:1424-1429; discussion 1430.

15. Raghoebar GM, Meijndert L, Kalk WW, Vissink A. Morbidity of mandibular bone harvesting: a comparative study. Int J Oral Maxillofac Implants 2007: 22:359-365.

16. Raghoebar GM, Louwerse C, Kalk WW, Vissink A. Morbidity of chin bone harvesting. Clin Oral Implants Res 2001: 12: 503-507.

17. Connolly JF, Guse R, Tiedeman J, Dehne R. Autologous marrow injection as a substitute for operative grafting of tibial nonunions. Clin Orthop Relat Res 1991: 259-270.

18. Connolly JF, Guse R, Tiedeman J, Dehne R. Autologous marrow injection for delayed unions of the tibia: a preliminary report. J Orthop Trauma 1989: 3: 276-282.

19. Wongchuensoontorn C, Liebehenschel N, Schwarz U, Schmelzeisen R, Gutwald R, Ellis E 3rd, Sauerbier S. Application of a new chair-side method for the harvest of mesenchymal stem cells in a patient with nonunion of a fracture of the atrophic mandible--a case report. J Craniomaxillofac Surg 2009: 37: 155-161.

20. Gutwald R, Haberstroh J, Kuschnierz J, Kister C, Lysek DA, Maglione M, Xavier SP, Oshima T, Schmelzeisen R, Sauerbier S. Mesenchymal stem cells and inorganic bovine bone mineral in sinus augmentation: comparison with augmentation by autologous bone in adult sheep. Br J Oral Maxillofac Surg 2010: 48: 285-290.

21. Sauvigne T, Fusari JP, Monnier A, Breton P, Freidel M. [The retromolar area, an alternative for the mandibular symphisis graft in implant surgery: quantitative and qualitative analysis of 52 samples]. Rev Stomatol Chir Maxillofac 2002: 103: 264-268.

22. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999: 284: 143-147.

23. Pinheiro JC & Bates DM. Mixed-effects models in S and S-PLUS. New York: Springer, 2000.

24. Wallace SS, Froum SJ. Effect of maxillary sinus augmentation on the survival of endosseous dental implants. A systematic review. Ann Periodontol. 2003: 8:328-343.

25. Del Fabbro M, Testori T, Francetti L, Weinstein R. Systematic review of survival rates for implants placed in the grafted maxillary sinus. Int J Periodontics Restorative Dent 2004: 24: 565-577.

26. Aghaloo TL, Moy PK. Which hard tissue augmentation techniques are the most successful in furnishing bony support for implant placement? Int J Oral Maxillofac Implants 2007: 22: 49-70.

27. Terheyden H, Jepsen S, Möller B, Tucker MM, Rueger DC. Sinus floor augmentation with simultaneous placement of dental implants using a combination of deproteinized bone xenografts and recombinant human osteogenic protein-1. A histometric study in miniature pigs. Clin Oral Implants Res 1999: 10: 510-521.

28. Araújo M, Linder E, Wennström J, Lindhe J. The influence of Bio-Oss Collagen on healing of an extraction socket: an experimental study in the dog. Int J Periodontics Restorative Dent 2008: 28:123-135.

29. Yildirim M, Spiekermann H, Handt S, Edelhoff D. Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: a histologic and histomorphometric clinical study in humans. Int J Oral Maxillofac Implants 2001: 16: 23-33.

30. Yildirim M, Spiekermann H, Biesterfeld S, Edelhoff D. Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood. A histologic and histomorphometric study in humans. Clin Oral Implants Res 2000: 11: 217-229.

31. Thorwarth M, Schlegel KA, Wehrhan F, Srour S, Schultze-Mosgau S. Acceleration of de novo bone formation following application of autogenous bone to particulated anorganic bovine material in vivo. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006: 101: 309-316.

32. Sauerbier S, Stubbe K, Maglione M, Haberstroh J, Kuschnierz J, Oshima T, Xavier SP, Brunnberg L, Schmelzeisen R, Gutwald R. Mesenchymal stem cells and bovine bone mineral in sinus lift procedures--an experimental study in sheep. Tissue Eng Part C Methods 2010: 16:1033-1039.

33. Peister A, Mellad JA, Larson BL, Hall BM, Gibson LF, Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood 2004: 103:1662-1668.

34. Sauerbier S, Stricker A, Kuschnierz J, Bühler F, Oshima T, Xavier SP, Schmelzeisen R, Gutwald R. In vivo comparison of hard tissue regeneration with human mesenchymal stem cells processed with either the FICOLL method or the BMAC method. Tissue Eng Part C Methods 2010: 16: 215-223.

35. Dennis JE, Esterly K, Awadallah A, Parrish CR, Poynter GM, Goltry KL. Clinical-scale expansion of a mixed population of bone-marrow-derived stem and progenitor cells for potential use in bone-tissue regeneration. Stem Cells 2007: 25: 2575-2582.

36. Shayesteh YS, Khojasteh A, Soleimani M, Alikhasi M, Khoshzaban A, Ahmadbeigi N. Sinus augmentation using human mesenchymal stem cells loaded into a beta-tricalcium phosphate/hydroxyapatite scaffold. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008: 106: 203-209.

37. Ueda M, Yamada Y, Ozawa R, Okazaki Y. Clinical case reports of injectable tissue-engineered bone for alveolar augmentation with simultaneous implant placement. Int J Periodontics Restorative Dent 2005: 25: 129-137.

38. Ueda M, Yamada Y, Kagami H, Hibi H. Injectable bone applied for ridge augmentation and dental implant placement: human progress study. Implant Dent 2008: 17: 82-90.

39. Fehrer C, Brunauer R, Laschober G, Unterluggauer H, Reitinger S, Kloss F, Gülly C, Gassner R, Lepperdinger G. Reduced oxygen tension attenuates differentiation capacity of human mesenchymal stem cells and prolongs their lifespan. Aging Cell 2007: 6: 745-757.

40. Smiler D, Soltan M, Lee JW. A histomorphogenic analysis of bone grafts augmented with adult stem cells. Implant Dent 2007:16: 42-53.

41. Minamide A, Yoshida M, Kawakami M, Yamasaki S, Kojima H, Hashizume H, Boden SD. The use of cultured bone marrow cells in type I collagen gel and porous hydroxyapatite for posterolateral lumbar spine fusion. Spine 2005:30: 1134-1138.

42. Jung RE, Glauser R, Schärer P, Hämmerle CH, Sailer HF, Weber FE. Effect of rhBMP-2 on guided bone regeneration in humans. Clin Oral Implants Res 2003: 14: 556-568.

43. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoebar GM. Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. Clin Oral Implants Res 2011: 22: 251-258.

BMAC (1)

Chapter 5

Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or bone marrow concentrate.

A prospective randomized clinical trial.

This chapter is an edited version of the manuscript.

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Abstract

Aim: To assess whether differences occur in bone formation after maxillary sinus floor elevation surgery with bovine bone mineral (BioOss®) mixed with autogenous bone or autogenous bone marrow fraction enriched in mesenchymal stem cells (MSCs). The primary endpoint was percentage of new bone three months after the elevation procedure.

Material and Methods: In a randomized controlled split-mouth design, in 12 consecutive patients (age 60.8±5.9 years, range 48-69 years) needing reconstruction of their atrophic maxilla, a bilateral sinus floor augmentation procedure was performed. Randomly, on one site the augmentation procedure was performed with bovine bone mineral (BioOss®) seeded with mononuclear autogenous bone marrow fraction enriched in mesenchymal stem cells (MSCs) harvested from the posterior iliac crest (test group) while BioOss® mixed with autogenous bone (harvested from the retromolar area) was applied on the contra-lateral site (control group). On 14.8±0.7 weeks after the sinus floor elevation, biopsies from the reconstructed areas were taken at the spots were subsequently the endosseous implants were placed. The biopsies were histomorphometrically analyzed.

Results: Significantly more bone formation was observed in the test group (17.7%±7.3) when compared to the control group (12.0%±6.6; p=0.026). In both the test and control group, all implants could be placed with primary stability. In one patient not all biopsies contained BioOss®. This patient was excluded from analysis.

Conclusion: Autogenous bone marrow fraction enriched in mesenchymal stem cells (MSCs) seeded on BioOss® particles can induce the formation of a sufficient volume of new bone to enable reliable placement of implants within a time frame comparable to that of applying either solely autogenous bone or a mixture of autogenous bone and BioOss®. This technique could be an alternative to using autografts.

Introduction

Application of dental implants to support full dentures in edentulous patients has evolved into a viable prosthodontic alternative to conventional prostheses. However, implant procedures in the posterior maxilla often pose a problem due to an insufficient bone volume (1). This restriction is not reserved to edentulous patients, but also is often observed in partial dentate patients needing an implant-based prosthodontic reconstruction in the posterior region of the maxilla.

The lack of bone to enable reliable placement of implants in the posterior maxilla can be solved by a maxillary sinus floor elevation procedure using autogenous bone, bone substitutes or a mixture of autogenous bone and bone substitutes as grafting materials (2). During this elevation procedure, the space created between the residual maxillary ridge and the elevated Schneiderian membrane is filled with a grafting material. This way, a bone volume is created that may allow for implant placement, either simultaneously with the elevation procedure when the residual ridge allows for primary implant stability or at a second stage after healing of the grafted site.

Regarding the various augmentation materials that have been used for a sinus elevation procedure, autogenous bone, with its osteogenic, osteoinductive and osteoconductive properties, is still by many surgeons considered the ideal grafting material (3). However, donor site morbidity is a major problem accompanying bone-harvesting techniques and puts the patient at an inconvenience that probably can be reduced or even be avoided when using synthetic bone substitutes (1). To surpass donor site morbidity, bone substitutes as calcium phosphates, β-tricalcium phosphates (Cerasorb®) (1, 2) bioactive glass particles (5, 6), xenogenic substitutes as bovine hydroxyapatites (BioOss®) (2, 7) and allogenic substitutes as demineralized freeze dried human bone (8) have commonly been proposed as and shown to be adequate alternatives for autologous bone. A major drawback of these substitutes is the rather long healing time that is needed before implants can be placed (7). Moreover, these substitutes are not very suitable to be used as a sole grafting material for large reconstructions. In addition, as clinicians often are looking for tools to speed up healing, the effect of using platelet rich plasma (PRP) has been studied aiming to accelerate bone regeneration as it has been speculated that growth factors within PRP could enhance healing of the grafts and counteract resorption after augmentation (9, 10). However, Raghoebar et al. (11) and Schaaf et al. (12) showed that no relevant differences in healing of soft tissues and bone existed between sites reconstructed with autogenous bone and autogenous bone mixed with PRP.

The combination of autogenous bone and bovine bone material has been investigated in previous histological sinus floor elevation studies. Yildirim et al. (13) examined sinus floor elevation procedures with only BioOss® in 15 sinus of 11 patients and found 14.7± 5.0% new bone formation after a healing time of 6.8 months. In another study Yildirim et al. (14) evaluated the bone formation in sinus floor elevations performed with a mixture of autogenous bone and BioOss® in 13 sinus floor elevations and reported 18.9±6.4 % new bone formation

after a 7.1 months healing period. Therefore, the addition of autogenous bone to the bovine bone material yielded an advantage of 4.2 % new bone, which can be translated in a delay in the healing time of a couple of months when applying solely BioOss®.
In recent animal studies it has been shown that seeding BioOss® with autogenous bone marrow fraction enriched in mesenchymal stem cells (MSCs) derived from concentrated non-mineralized tissue may result in bone forming kinetics comparable to bone forming kinetics in a region solely reconstructed with autogenous bone (15). MSCs were shown to differentiate to osteoblasts when being introduced into an environment prone to formation of bone. In addition, in an in vitro study osteoblast-like cells were cultured on various alloplastic biomaterials used for augmentation and for reconstruction of bone defects in dental and craniomaxillofacial surgery (16). The latter study revealed that osteoblast like cells attach to BioOss® and offer suitable growth and proliferation conditions. Furthermore, Gutwald et al. (15) compared in a sheep model the osteogenic potential of mononuclear cells harvested from the iliac crest combined with bovine bone mineral to autogenous cancellous bone alone. Bilateral sinus floor augmentations were carried out. Histomorphometric analysis of biopsies taken after 8 and 16 weeks after the augmentation procedure revealed the bone forming potential of mononuclear cells, including the autogenous bone marrow fraction enriched in mesenchymal stem cells in combination with BioOss® as biomaterial (15). Furthermore, Herten et al. (17) evaluated the influence of different bone substitutes (BioOss®) on the viability of human bone marrow mesenchymal stem cells in vitro and concluded that hydroxyapatite (BioOss®) support cell viability and allow cell proliferation. The promising results from in vitro and animal studies (15) stimulated us to perform a study in human. In this randomized controlled trial (RCT) it was assessed whether differences in bone formation occurred after maxillary sinus floor elevation surgery with either autogenous bone in combination with BioOss® or bone marrow fraction enriched in MSCs in combination with BioOss®. The primary endpoint was percentage of new bone three months after the elevation procedure.

Materials and methods

This study is a joint study between the University of Freiburg and the University Medical Center Groningen. The protocol was approved by the ethics committees and the study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients. All patients were treated according to the protocol for reconstruction of bony defects with a mixture of bone substitutes and autogenous bone marrow fraction enriched in MSCs developed in Freiburg (15). All patients were treated with a split-mouth design.

Edentulous patients older than 18 years with need of dental implant placement in the posterior maxilla were eligible for the study if they had a maximum of 4 mm residual height of the alveolar ridge at either site of the maxilla. In addition, the patients had to be able to comply

Table 1. Radiological bone height of the residual alveolar processus at the implant site (in mm)

No patient	Bone height at baseline in mm (right side)	Bone height at baseline in mm (left side)
1	3	2
2	2	2
3	2	2
4	2	2
5	2	3
6	2	2
7	2	2
8	2	1
9	2	2
10	2	2
11	2	3
12	2	3
Mean	**2.1±0.3**	**2.2±0.6**

with study related procedures including returning for follow-up examinations, exercising good oral hygiene and being able to understand the nature of the proposed surgery. Exclusion criteria were (1) smoking, (2) history of malignancy, radiotherapy or chemotherapy, (3) pregnancy or nursing, (4) medication, treatment or disease, which may have an effect on bone turnover, bone or non-mineralized tissue metabolism, and (5) allergy to collagen.

Patients

The patients were referred to the Department of Oral and Maxillofacial Surgery of the University Medical Center Groningen because of insufficient retention of their upper denture related to a severely resorbed maxilla selected on basis of the following inclusion criteria:

- severely resorbed maxilla (class V-VI, (18)) with reduced stability and retention of the upper denture;
- comparable bone height between the maxillary sinus and top of the maxilla on both sites;
- class IV bone quality (19);
- edentulous period of at least one year;
- no history of radiotherapy in the head and neck region;
- no history of reconstructive, pre-prosthetic surgery or previous oral implantology.

Orthopantomograms, lateral cephalograms, and postero-anterior oblique radiographs were

made to assess the height of the maxillary alveolar bone, the dimensions of the maxillary sinus, and the antero-posterior relationship of the maxilla to the mandible. The radiographs were also screened for sinus pathology.

In 12 consecutive patients (age 60.8±5.93 years, range 48-69 years) needing reconstruction of their atrophic maxilla and who fulfilled the inclusion criteria, a bilateral sinus floor augmentation procedure was performed (split-mouth design). The mean vertical height of the alveolar bone on the orthopantomogram between the most caudal part of the maxillary sinus and the oral cavity were in the premolar and molar region on the right 2.1±0.3 mm and on the left site 2.2±0.6 mm, respectively (Table 1). Randomly, performed by envelopes, on one site the augmentation procedure was performed with bovine bone mineral (BioOss®, Geistlich Biomaterials, Wolhusen, Switzerland) seeded with bone marrow fraction enriched in MSCs harvested from the posterior iliac crest (test group) and BioOss® combined with autologous bone on the contra-lateral site (control group).

Harvesting of bone marrow concentrate

The patients were treated under general anesthesia. The pelvic bone was punctured about 2 cm laterocaudally from the superior posterior iliac spine with a bone marrow biopsy needle. With a 60 ml syringe flushed with heparin solution (Heparin-Natrium, 10.000 U/ml, diluted with NaCl to 1000 U/ml, both B. Braun, Melsungen, Germany) and then filled with 8 ml of citric acid (BMAC-Kit, Harvest Technologies Corporation, Plymouth, MA, USA), 52 ml of non-mineralized tissue was collected. According to the instructions of the manufacturer non-mineralized tissue was isolated directly in the operating room by using the BMAC system (Bone Marrow Procedure Pack, Harvest Technologies Corporation, Plymouth, MA, USA). The procedure of concentrating the bone marrow aspirates took about 15 minutes. For details of the selection procedure and characterization of the MSCs see Sauerbier et al. (20, 21). In these studies cells from the non-mineralized tissue concentrate were amplified and differentiated into chondrogenic, adipogenic and osteogenic cell lineages according to the methods according to Pittenger et al. (22). The cultured MSCs could be differentiated successfully into adipocytes, chondrocytes and osteoblasts. Flowcytrometic analysis showed a distinct population of CD 34 and CD 45 negative cells which were positive for CD44 and CD73.

3 ml of non-mineralized tissue concentrate and 1 ml autologous thrombine produced from venous blood (Thrombin Kit, Harvest Technologies Corporation, Plymouth, MA, USA) were applied on 2 g of BBM (BioOss® 1-2mm, Geistlich Pharma AG, Wolhusen, Switzerland).

The bone harvesting was performed by the same surgeon from the same region with the same method. The bone was particulated with a bone mill to a grain size of 1-2mm. 30 % of bone was mixed with 70 % of BBM (BioOss® 1-2mm, Geistlich Pharma AG, Wolhusen, Switzerland). The relation was determined by volumetric measurement.

Sinus augmentation and implant placement procedure

An osteotomy was prepared in the lateral wall of the maxillary sinus using the surgical procedure described by Raghoebar et al. (23) after a pedicled mucoperiostal flap was raised to expose the lateral wall of the maxillary sinus. The floor of the maxillary sinus (test site) was augmented with BioOss® (0.25-1mm, Geistlich Pharma AG, Wolhusen, Switzerland) and enriched with mononucleated cells in thrombin according to the method of Gutwald et al. (15). Autologous thrombine produced from venous blood (Thrombin Kit, Harvest Technologies Corporation, Plymouth, MA, USA) was used to clot the non-mineralized tissue concentrate.
The control site was augmented with a mixture of 70% biomaterial and 30% autogenous bone harvested from the retromolar area as described by Capelli (24). In addition, the width of the superior alveolar process had to be reconstructed with mandibular bone in 10 out of the 12 patients at both sites (25, 26). All bone grafts were harvested from the retromolar region. The grafts were fixed with titanium screws. Moreover, a guide on which the planned position of the implants was marked, was used to be certain that implants will be placed in reconstructed areas.

A collagen membrane (Bio-Gide®, Geistlich Pharma AG, Wolhusen, Switzerland) was used to cover the facial sinus wall defect on the surface of both grafted sites. The mucoperiostal flap was replaced and wound closure was performed by using resorbable suture material Vicryl 4.0 (Ethicon, Norderstedt, Germany). In the evening of the operating day the patients were discharged from the hospital according to the outpatient protocol. All patients received broad spectrum antibiotics, starting one hour preoperatively (intravenously) and continued orally for two days after surgery. Postoperatively the patients received a 0.2% chlorhexidine mouth rinse (1 minute, 5 times daily) for 2 weeks. One month postoperatively, the edentulous patients were allowed to wear dentures if possible, after relining them in the operated areas with a soft liner.

After a healing period of 13-16 weeks, the implant placement procedure was performed. A surgical template was used. Using the template, biopsies were taken with a trephine burr (Ø 2.6mm, 16mm length; Gebr. Brasseler GmbH & Co. KG, Lemgo, Germany) from the marked positions on the surgical template. On the same spot the endosseous implants were placed. The implants were inserted at the biopsy locations after widening these holes to the required dimensions using the standard burrs for the implant system chosen. The right position of the biopsy was confirmed later by the biomaterial (BBM) content which is clearly distinguishable in the histology. So the biopsies which contained BBM which was used in both groups must have been from the augmented area. In all cases the bone volume was sufficient. Three months after insertion the prosthetic construction was fabricated.

Histological evaluation

The reference area for the histomorphometrical evaluation was the entire area in the biopsy above the old bone of the sinus floor. Values measured in % of the examined area were taken for biomaterial, old bone and newly formed bone. The value for non-mineralized tissue was gained by subtracting the values of biomaterial, old bone and newly formed bone from the total evaluated area.

The burrs with the bone biopsies inside were fixed in formalin for 48 hours, rinsed in water and dehydrated in serial steps of alcohol (70%, 80%, 90% and 100%) remaining for 3 days in each concentration. After dehydration the samples were infiltrated with resin (Technovit 7200 VLC, Heareus Kulzer, Hanau, Germany) for 2 weeks. The resin was polymerized in a UV light chamber for 10 hours. After the hardening two sections of 300 µm to 400 µm thickness and parallel to the axis of the burr were cut with a diamante micro saw (Microslice, IBS, Cambridge, GB). The sections were placed on an acrylic slide (Maertin, Freiburg, Germany) and reduced to a thickness of approximately 100 µm on a rotating grinding plate (Struers, Ballerup, Denmark). The specimens were stained with Azur II and Pararosanilin which allowed for a differentiation between BioOss® particles, preexisting and newly formed bone (Figures. 1A, 2A). Histomorphometric examination was done with a light microscope (Axiovert 135, Zeiss, Kochern, Germany) (20). The BioOss® particles were marked and the new formed bone around the particles was measured (Figures 2A, 2B). The marking and measurements were performed with the computer software AnalySIS[D] Soft Imaging system (Olympus Europa GmbH, Hamburg, Germany). The histologists were blinded to the samples' groups throughout the histomorphometrical analysis.

Statistical analysis

For the parameters NewBone (new bone formation), BioOss® (Biomaterial) and Marrows (marrow space), values were expressed in % of the evaluated area. For statistical analysis a nonparametric Wilcoxon test for paired group data was used. 11 out of 12 patients were included for analysis as in one patient it was shown that at one site no biopsies were available from an augmented region. A $p<0.05$ is considered as a significant result.

Results

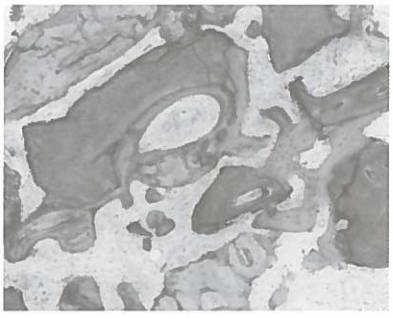
Healing was uneventful. Loss of bone particles through the nose was not observed. A minor incision breakdown occurred in the first week in 1 patient at the test site. This patient was put on a regimen of rinsing with a 0.12% w/v chlorhexidine mouth rinse 4 times daily. The dehiscence (5mm x 5mm) healed uneventful within two weeks.

Figure 1 Biopsy taken from the experimental side at 3.1 months after grafting. In this specimen 16.2% of new bone was present. The newly formed bone lamellae connected the biomaterial particles and stabilized the grafted complex. The grafted biomaterial with newly formed bone was well integrated in the surrounding host bone. There were no signs of an inflammatory reaction. The grafted bone shows signs of resorption and new bone formation, both are signs of active bone remodeling.

A. Azur II and pararosanilin staining.

B. View as used for histomorphometrical analysis.

new formed bone
BioOss®



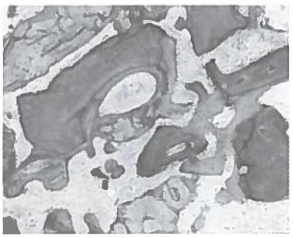
C. Newly formed bone is clearly deposited around the BioOss® particles. No indication of BioOss® resorbtion was observed, at 5x magnification.

Figure 2 Biopsy taken from the control side at 3.4 months after grafting. In this specimen 11.8% of new bone was present. The newly formed bone lamellae connected the biomaterial particles and stabilized the grafted complex. The grafted biomaterial with newly formed bone was well integrated in the surrounding host bone. There were no signs of an inflammatory reaction. The grafted bone shows signs of resorption and new bone formation, both are signs of active bone remodeling.

A. Azur II and pararosanilin staining.

B. View as used for histo-morphometrical analysis.

new formed bone;
BioOss®;
autogenous bone.



C. Newly formed bone is deposited near BioOss® particles and autogenous bone.
No indication of BioOss® resorbtion was observed, while the autogenous bone is in the process of being resorbed and replaced by newly formed bone, at 5x magnification.

All 12 patients were treated with a bilateral sinus floor augmentation procedure, but the results of the biopsies taken from one sinus (control site) of one patient were not included in this analysis as histological examination revealed that these biopsies were not taken from an augmented location. No BioOss® could be identified in these biopsies (internal control as at both control and test sites BioOss® was applied). By design (split-mouth approach), this patient was removed from the comparisons and thus the data set to be analyzed was composed of data from the 11 patients in whom biopsies (all containing BioOss® particles) were available from both the control and test sites. Per patient four to six cylindrical bone biopsies were available (two to three of each site). All biopsies had been taken within the 13 to 16 weeks post-augmentation period (mean 14.8±0.7 weeks, range 13.3-15.8 weeks). In table 2 average values for percentage of newly formed bone, BioOss® and marrow space of the biopsies taken at a particular site are shown.

Histology

The newly formed bone lamellae surrounded the biomaterial particles and stabilized the grafted complex. The grafted material (biomaterial with newly formed bone) was well integrated in the surrounding host bone (Figure 1).
Vital bone tissue containing osteocytes inside the bone lacunae were observed in the newly formed osseous lamellae. The biomaterial could be easily identified by its size, shape and colour in comparison to newly formed bone or the pre-existing local bone. Newly formed bone appeared darker than the BioOss® particles in the Azur II Pararosanilin staining (Figures 1 and 2). Blood vessels could be detected throughout the specimens showing that the blood supply is ensured throughout the whole augmentate. There were no signs of an inflammatory reaction.

Histomorphometry

New bone formation. Significantly more new bone had been formed at the reconstructed areas at time of implant in the test group when compared to the control group (primary end point; Table 2; p=0.026).
BioOss®. At three months post sinus floor evaluation surgery, the percentage of BioOss® present in the biopsies taken from the test and control sites was comparable (Table 2; p=0.722).
Marrow space. The percentage of the biopsies occupied by a marrow space was comparable between the test and control specimen (Table 2; p= 0.859).

Implants

Comparison of the clinical features at the test and control revealed no differences with regard to wound healing and complications during or post surgery. In all augmented regions,

Table 2. Percentages of newly formed bone, BioOss® and marrow space at time of implant placement in the reconstructed areas.

Patient	% New Bone		% BioOss		% Marrow space	
	Test	Control	Test	Control	Test	Control
1	13.6	13.7	24.3	29.8	62.1	52.9
2	30.1	14.7	12.3	25.6	57.7	55.1
3	20.9	11.8	36.9	35.4	42.2	45.5
4	32.1	27.1	13.4	23.9	52.5	48.5
5	12.2	8.4	29.3	20.1	58.7	62.1
6	13.9	16.1	37.1	28.5	48.9	50.7
7	11.7	17.5	42.1	9.8	46.1	71.7
8	*	*	*	*	*	*
9	13.7	6.2	31.3	32	54.2	51.8
10	21.3	11.2	36.5	35.4	47.3	45.5
11	13.7	4	32.1	25.1	54.2	56.6
12	24.3	13.2	23.3	25.2	52.5	54.4
Mean±SD	17.7(±7.3)	12.2(±6.6)	28.96(±9.7)	26.4(±6.97)	52.4(±5.9)	54.6(±7.5)
Median	13.9	13.2	31.3	25.6	52.5	52.9
P- value	p= 0.026		p= 0.722		p= 0.859	

* Patient 8 was not included in the results as biopsies from the test site did not contain BioOss® particles

implants could be installed with primary stability. A total of 66 nonsubmerged one-piece implants (ITI Straumann®, Institut Straumann, Waldenburg, Switzerland) was placed in the augmented maxillae.

Before the prosthetic phase, 3 implants (two patients) were mobile on the test site and had to be removed. In one patient the suprastructure could be made on the remaining two implants on that site, while in the other patient the two lost implants were replaced. Healing was uneventful and also this patient could be supplied with an adequately functioning implant-supported maxillary overdenture.

Discussion

Currently, the most reliable and well studied grafting materials to perform sinus floor augmentation surgery are autogenous bone and mixtures of autogenous bone with BioOss® (2). It is questionable, however, whether adding autogenous bone to biomaterials as BioOss®

is necessary. Our randomized, controlled split-mouth study showed that as an augmentation material to be used for a sinus floor augmentation procedure, BioOss® seeded with bone marrow fraction enriched in MSCs was shown to be superior to BioOss® mixed with autogenous bone with respect to bone formation three to four months after surgery.

As shown in our study, adding mononuclear cells, including the mesenchymal stem cell fraction, to BioOss®, can lead to more new bone formation compared to BioOss® combined with autologous bone. These results are supported by the results from various (animal) studies (13, 15, 17, 21). They showed in a sheep model the osteogenic potential of mononuclear cells and the bone forming potential of mononuclear cells, including the bone marrow fraction enriched in MSCs in combination with BioOss® as biomaterial. Pieri et al. (27) investigated whether mesenchymal bone marrow fraction enriched in MSCs and PRP seeded on a fluorhydroxyapatite scaffold can improve bone formation and bone-to-implant contact in maxillary sinus grafting. They showed that sinus augmentation with bone marrow fraction enriched in MSCs may enhance bone formation and osseointegration of dental implants in minipigs. Also McAllister et al. (28) showed that treatment with bone marrow fraction enriched in MSCs has a positive effect on bone formation. The purpose of their case series was to evaluate the bone formation following sinus-augmentation procedures using an allograft cellular bone matrix containing native bone marrow fraction enriched in MSCs. Next to the promising results from the application of bone marrow fraction enriched in MSCs, also other studies challenged whether autogenous bone still has to be considered as the grafting material of first choice. E.g., Hallman et al. (2) studied the graft/titanium implant interface in maxillary sinus augmented with autogenous bone, bovine hydroxyapatite, or an 80-20% mixture of bovine hydroxyapatite and autogenous bone. They reported no significant differences in healing of the augmented sites between the three groups after six to nine months. In addition, Zerbo et al. (1) and Szabo et al. (4) compared the applicability of autogenous bone and β-tricalcium phosphate for sinus floor elevation surgery in a split mouth design. In both studies it was concluded that on the long run (i.e., >6 months) there was no significant difference in healing of the augmented sites, although Zerbo et al. (1) mentioned that the rate of bone formation was delayed by approximately six months in the test site when compared to the site reconstructed with to autogenous bone. Finally, also bioglass, a material that has been shown to be able to directly chemically bond to bone, has been shown a potentially applicable grafting material for reconstructive procedures. When applied in the size range of 300 to 355 µm, bioglass showed osteoconductive properties (5, 6).

As mentioned in the previous paragraph, it is commonly known that a bovine bone mineral as BioOss® is in need of a longer healing period than autogenous bone before implants can be placed (7). A healing time of 6 months before implant placement is recommended for BioOss®. With regard to our study, we were not allowed to compare BioOss® in combination with bone marrow fraction enriched in MSCs to treatment with BioOss® alone because the ethics-committee did not allow biopsy and implant placement after 13-16 weeks in a BioOss®-only-group. Adding mononuclear cells derived from a non-mineralized tissue as-

pirate to BioOss® has been shown to result in bone forming kinetics comparable to autogenous bone alone (16). In our study, 3.8 months after treatment all mixtures (control and test group) showed more new bone formation than other studies after a healing period of six up to nine months in which patients were treated with BioOss® alone (2, 29). Furthermore, our results are comparable with those achieved with BioOss® alone at later healing time points, 6 to 8 months (29). In the latter randomized, controlled investigation, the authors histomorphometrically evaluated the formation of vital bone following bilateral grafting with two different materials--Puros, a mineralized cancellous bone allograft (MCBA) and BioOss® at 26 to 32 weeks. Histomorphometric analysis of 10 MCBA cores and 9 BioOss® cores revealed average vital bone content of 28.25% and 12.44%, respectively. Significantly more bone was formed in the MCBA sites after a healing time of 6 up to 8 months. The use of a grafting material to perform a sinus lift procedure even may become questionable as a recent study has demonstrated that the mere lifting of the sinus mucosal lining and simultaneous placement of implants also can result in bone formation (30). However, currently this technique only is applied for conditions allowing for sufficient primary stability of implants during placement and a sufficient width of the alveolar crest but not for reconstruction in horizontal and vertical direction, which was not the case in the subjects included in our trial. Moreover, for evidence whether this treatment indeed reliably will result in induction of bone growth, well designed studies have to be carried out in the future. Finally, in all our cases we had to both increase the height as to widen the posterior maxillary ridge.

From this study it is concluded that bone marrow fraction enriched in MSCs derived from an aspirate of the posterior iliac crest seeded on BioOss® particles can induce the formation of a sufficient volume of new bone to enable reliable placement of implants within a time frame comparable to that of applying either solely autogenous bone or a mixture of autogenous bone and BioOss®. This technique could be an alternative to auto-grafts, in particular by surpassing their inherent donor site morbidity.

References

1. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM, Burger EH. Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants 2004: 15: 724-732.

2. Hallman M, Sennerby L, Lundgren S. A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. Int J Oral Maxillofac Implants 2002: 17: 635-643.

3. Hallman M, Thor A. Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. Periodontol 2000 2008: 47: 172-192.

4. Szabo G, Huys L, Coulthard P, Maiorana C, Garagiola U, Barabas J, Nemeth Z, Hrabak K, Suba Z. A prospective multicenter randomized clinical trial of autogenous bone versus beta-tricalcium phosphate graft alone for bilateral sinus elevation: histologic and histomorphometric evaluation. Int J Oral Maxillofac Implants 2005: 20: 371-381.

5. Tadjoedin ES, de Lange GL, Holzmann PJ, Kulper L, Burger EH. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. Clin Oral Implants Res 2000: 11: 334-344.

6. Tadjoedin ES, de Lange GL, Lyaruu DM, Kuiper L, Burger EH. High concentrations of bioactive glass material (BioGran) vs. autogenous bone for sinus floor elevation. Clin Oral Implants Res 2002: 13: 428-436.

7. John HD, Wenz B. Histomorphometric analysis of natural bone mineral for maxillary sinus augmentation. Int J Oral Maxillofac Implants 2004: 19: 199-207.

8. Wallace SS, Froum SJ, Cho SC, Elian N, Monteiro D, Kim BS, Tarnow DP. Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: histomorphometric and clinical analyses. Int J Periodontics Restorative Dent 2005: 25: 551-559.

9. Thor A, Wannfors K, Sennerby L, Rasmusson L. Reconstruction of the severely resorbed maxilla with autogenous bone, platelet-rich plasma, and implants: 1-year results of a controlled prospective 5-year study. Clin Oral Implants Res 2005: 7: 209-220.

10. Thor A, Franke-Stenport V, Johansson CB, Rasmusson L. Early bone formation in human bone grafts treated with platelet-rich plasma: preliminary histomorphometric results. Int J Oral Maxillofac Surg 2007: 36: 1164-1171.

11. Raghoebar GM, Schortinghuis J, Liem RS, Ruben JL, van der Wal JE, Vissink A. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clin Oral Implants Res 2005: 16: 349-356.

12. Schaaf H, Streckbein P, Lendeckel S, Heidinger K, Gortz B, Bein G, Boedeker RH, Schlegel KA, Howaldt HP. Topical use of platelet-rich plasma to influence bone volume in maxillary augmentation: a prospective randomized trial. Vox Sang 2008: 94: 64-69.

13. Yildirim M, Spiekermann H, Biesterfeld S, Edelhoff D.Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood. A histologic and histomorphometric study in humans. Clin Oral Implants Res 2000: 11: 217-229.

14. Yildirim M, Spiekermann H, Handt S, Edelhoff D. Maxillary sinus augmentation with the xenograft Bio-Oss and autogenous intraoral bone for qualitative improvement of the implant site: a histologic and histomorphometric clinical study in humans. Int J Oral Maxillofac Implants 2001: 16: 23-33.

15. Gutwald R, Haberstroh J, Kuschnierz J, Kister C, Lysek DA, Maglione M, Xavier SP, Oshima T, Schmelzeisen R, Sauerbier S. Mesenchymal stem cells and inorganic bovine bone mineral in sinus augmentation: comparison with augmentation by autologous bone in adult sheep. Br J Oral Maxillofac Surg 2010: 48: 285-290.

16. Schmitt SC, Wiedmann-Al-Ahmad M, Kuschnierz J, Al Ahmad A, Huebner U, Schmelzeisen R, Gutwald R. Comparative in vitro study of the proliferation and growth of ovine osteoblast-like cells on various alloplastic biomaterials manufactured for augmentation and reconstruction of tissue or bone defects. J Mater Sci Mater Med 2008: 19: 1441-1450.

17. Herten M, Rothamel D, Schwarz F, Friesen K, Koegler G, Becker J. Surface- and nonsurface-dependent in vitro effects of bone substitutes on cell viability. Clin Oral Investig 2009: 13: 149-155.

18. Cawood JJ & Howell RA. A classification of edentulous jaws. Int J Oral Maxillofac Surg 1988: 17: 232–236.

19. Lekholm U & Zarb GA. Patient selection and preparation. In: Bra°nemark, P.-I., Zarb, G. A. & Albrektsson, T. (eds). Tissue-Integrated Prostheses. Chicago: Quintessence Publishing Co. 1986: 199–209.

20. Sauerbier S, Stricker A, Kuschnierz J, Bühler F, Oshima T, Xavier SP, Schmelzeisen R, Gutwald R. In vivo comparison of hard tissue regeneration with human mesenchymal stem cells processed with either the FICOLL method or the BMAC method. Tissue Eng Part C Methods 2010: 16: 215-223.

21. Sauerbier S, Stubbe K, Maglione M, Haberstroh J, Kuschnierz J, Oshima T, Xavier SP, Brunnberg L, Schmelzeisen R, Gutwald R. Mesenchymal stem cells and bovine bone mineral in sinus lift procedures--an experimental study in sheep. Tissue Eng Part C Methods 2010: 16: 1033-1039.

22. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. Science 1999: 284: 143-147.

23. Raghoebar GM, Timmenga NM, Reintsema H, Stegenga B, Vissink A. Maxillary bone grafting for insertion of endosseous implants: results after 12-124 months. Clin Oral Implants Res 2001: 12: 279-286.

24. Capelli M. Autogenous bone graft from the mandibular ramus: a technique for bone augmentation. Int J Periodontics Restorative Dent 2003: 23: 277-285.

25. Raghoebar GM, Meijndert L, Kalk WW, Vissink A. Morbidity of mandibular bone harvesting: a comparative study. Int J Oral Maxillofac Implants 2007: 22: 359-365.

26. Raghoebar GM, Slater JJ, Hartog L, Meijer HJ, Vissink A. Comparison of procedures for immediate reconstruction of large osseous defects resulting from removal of a single tooth to prepare for insertion of an endosseous implant after healing. Int J Oral Maxillofac Surg 2009: 38: 736-743.

27. Pieri F, Lucarelli E, Corinaldesi G, Iezzi G, Piattelli A, Giardino R, Bassi M, Donati D, Marchetti C. Mesenchymal stem cells and platelet-rich plasma enhance bone formation in sinus grafting: a histomorphometric study in minipigs. J Clin Periodontol 2008: 35: 539-546.

28. McAllister BS, Haghighat K, Gonshor A. Histologic evaluation of a stem cell-based sinus-augmentation procedure. J Periodontol 2009: 80: 679-686.

29. Froum SJ, Wallace SS, Elian N, Cho SC, Tarnow DP. Comparison of mineralized cancellous bone allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: histomorphometry at 26 to 32 weeks after grafting. Int J Periodontics Restorative Dent 2006: 26: 543-551.

30. Lundgren S, Cricchio G, Palma VC, Salata LA, Sennerby L. Sinus membrane elevation and simultaneous insertion of dental implants: a new surgical technique in maxillary sinus floor augmentation. Periodontol 2000 2008: 47: 193-205.

Chapter 6

Maxillary sinus floor elevation with bovine bone material combined with autogenous bone or bone marrow concentrate. A one year follow-up on implant survival and clinical performance.

Test of principle on implant survival and clinical performance.

This chapter is an edited version of the manuscript.

Rickert D, Vissink A, Slot JWA, Sauerbier S, Meijer HJA, Raghoebar GM.
Submitted

Abstract

Objectives To assess implant survival and one-year clinical performance regarding implants placed in the posterior maxilla that had been subjected to maxillary sinus floor elevation surgery with bovine bone mineral (BioOss®) mixed with an autogenous bone marrow concentrate or autogenous bone.

Material and methods In a randomized, controlled split-mouth design, in 12 edentulous patients a bilateral sinus floor augmentation procedure was performed. Randomly, on one site the procedure was performed with BioOss® seeded with a bone marrow concentrate from the iliac crest which was rich in mesenchymal stem cells (test) while BioOss® mixed with autogenous bone was applied on the control site. Three months after augmentation, 66 Straumann Standard Implants® were placed. At baseline and twelve months after functional loading, implant survival, plaque-, gingival-, bleeding-indices, probing depth and peri-implant radiographic bone levels were assessed.

Results During osseointegration, three implants failed at the test site (2 patients), no implants failed at the control site, resulting in three months survival rates of 91% and 100%. No implants were lost after functional loading. Neither differences in soft tissue parameters nor in peri-implant bone loss were observed between control and test sites during follow up.

Conclusion After one year in function, no clinical relevant differences were observed regarding soft tissue parameters and peri-implant bone loss. However, implant survival rate tended to be lower in the test group.

Introduction

The application of dental implants to support prosthetic constructions has evolved into a viable alternative to conventional prosthetic procedures (1, 2), although implant procedures in the posterior maxilla often pose a problem due to insufficient pre-existent bone (3, 4). This restriction is not reserved to edentulous patients, but is also often observed in partial dentate patients needing an implant-based prosthodontic reconstruction in the posterior region of the maxilla.

An insufficient volume of bone to allow for reliable primary placement of implants can be solved by a maxillary sinus floor elevation procedure using autogenous bone, bone substitutes or a mixture of autogenous bone and bone substitutes as grafting materials (5). Currently, the most reliable and well studied grafting materials to perform sinus floor augmentation surgery are autogenous bone and mixtures of autogenous bone with bovine bone material (BioOss®, Geistlich Biomaterials, Wolhusen, Switzerland) (5, 6). It is questionable, however, whether adding autogenous bone to biomaterials as BioOss® is necessary as the morbidity of the procedure might be considerably less when no donor site is needed. It has been shown that a sinus floor augmentation procedure can be performed by just using bone substitutes without any additions (7). The analysis of Nkenke and Stelzle (7) included titanium implants with modified surfaces placed in sites with a mean residual bone height up to 6 mm and a lateral wall approach to the sinus. When applying bone substitutes, longer healing times were needed than when applying autogenous bone or a mixture of autogenous bone and a bone substitute, however. Also Rickert et al. (5) concluded that bone substitutes combined with autogenous bone provide a reliable alternative for autogenous bone as a sole grafting material to reconstruct maxillary sinus bony deficiencies, for supporting dental implants when allowing for a sufficient healing period. Both observations were also in line with data from animal studies from which Jensen et al. (8) concluded that there is a need for longer healing times when solely bone substitutes were used as well as that addition of autogenous iliac bone to Bio-Oss may accelerate not only bone regeneration, but also bone to implant contact during the early healing period compared to Bio-Oss. In other words when reconstructing larger defects and/or when aiming for shorter healing times bone substitutes have to be combined by autogenous bone.

In recent animal studies it has been shown that seeding BioOss® with mesenchymal stem cells (MSCs) derived from concentrated non-mineralized tissue may result in bone forming kinetics comparable to bone forming kinetics in a region solely reconstructed with autogenous bone. These promising results from in vitro and animal study (9) were stimulating to perform a study in human. In a randomized controlled trial it was assessed whether differences in bone formation occurred after maxillary sinus floor elevation surgery with BioOss® either combined with an autogenous bone marrow concentrate (enriched in MSCs) or autogenous bone. The primary endpoint was percentage of new bone formed at three months after the elevation procedure, i.e. at the time of implant placement (10, 11). From this trial it was concluded that MSCs derived from an aspirate of the posterior iliac crest

seeded on BioOss® particles indeed can induce the formation of a sufficient volume of new bone to allow for placement of implants within a time frame comparable to that of applying either solely autogenous bone or a mixture of autogenous bone and BioOss®. This time frame is considerably shorter than when only BioOss® is applied (5, 10-12). Adding MSCs to a bone substitute could be an alternative to the use of auto-grafts as a grafting material thereby reducing donor site morbidity (13, 14), particularly when larger grafts are needed. E.g., as the iliac crest is commonly used as the donor site for patients who need a bilateral, vertical maxillary sinus lift, replacement of autogenous bone by bone substitutes might considerably decrease the morbidity and discomfort of the grafting procedure from perspective of the patient. As the results from the above described trial were very promising, the next step should be to assess whether, after loading, dental implants placed in an area reconstructed with a MCSs enriched bone marrow concentrate perform at least as well as implants placed in an area reconstructed with a mixture of autogenous bone and BioOss® with respect to implant survival and condition of peri-implant tissues in particular. Therefore, the aim of the current study was to assess the one-year implant survival rate, a variety of peri-implant parameters and patients' satisfaction in a group of patients in a randomized, double-blind split mouth design. Implants were placed in an area reconstructed with a combination of BioOss® with a bone marrow concentrate enriched in MSCs on the one site and a mixture of BioOss® and autogenous bone on the other site.

Patients and methods

The study described in this paper is a clinical follow-up of 12 patients that were involved in the randomized, double-blind split mouth study of Rickert et al. (10) on bone formation in maxillary sinus reconstructed with a mixture of BioOss® and a bone marrow concentrate enriched in MSCs or with a mixture of BioOss® and autogenous bone. Briefly, in 12 consecutive patients (age 60.8±5.9 years, range 48-69 years) with maxillary denture problems, a bilateral sinus floor augmentation procedure of their atrophic maxillae was performed. Randomly, by envelopes, on one site the augmentation procedure was performed with BioOss® (Geistlich Biomaterials, Wolhusen, Switzerland) seeded with a bone marrow concentrate from the posterior iliac crest enriched in MSCs. The contra-lateral site was augmented with a mixture of 70% biomaterial (BioOss®) and 30% autogenous bone (harvested from the retromolar area of the mandible; control site) as commonly applied in reconstructive surgery (6). Three to four months after the sinus floor elevation, endosseous implants (Straumann Standard Implant®, Institut Straumann, Waldenburg, Switzerland) were placed. Depending on the available intermaxillary space and prosthodontic needs 2-3 implants were placed at each posterior maxillary side. Totally, 66 implants could be installed with primary stability in the augmented maxillae. After an osseointegration period of three months, the prosthetic construction, being an implant-supported overdenture, was fabricated according to the procedure described by Slot et al. (15).

This study is part of a joint study of the Universities of Freiburg and Mainz, and the University Medical Center Groningen (10, 11). In total 38 patients were evaluated, amongst which 12 edentulous patients needing bilateral sinus floor elevation surgery were treated in a randomized, controlled, split mouth design. The other 26 partial edentulous patients were in a random order unilaterally treated with either the control or the test method. The current study evaluated the treatment outcome in the 12 edentulous patients needing bilateral sinus floor elevation surgery.
The protocol was approved by the ethics committee and the study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all patients.

Implant survival

A surviving implant was defined as an implant in place at the time of assessment.

Clinical examination

All 12 patients were evaluated by one dentist (WS), who was blinded for the grafting procedures, after placing the suprastructures (baseline, T0) and twelve months after functional loading (T12). The following soft tissue measurements were done at both periods:

Plaque index (Silness and Loë) (16)
Score 0: no plaque.
Score 1: a film of plaque adhering to the free gingival margin and adjacent area of the implant/abutment. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the implant/abutment surface.
Score 2: moderate accumulation of soft deposits within the mucosal pocket, or on the implant/abutment and mucosal margin, which can be seen with the naked eye.
Score 3: abundance of soft matter within the gingival pocket and/or on the implant/abutment and mucosal margin.

Gingival index (Loë and Sillness) (17)
Score 0: absence of inflammation.
Score 1: mild inflammation; slight change in colour and little change in texture.
Score 2: moderate inflammation; moderate glazing, redness, oedema and hypertrophy; bleeding on pressure.
Score 3: severe inflammation; marked redness and hypertrophy; tendency towards spontaneous bleeding; ulceration.

Bleeding index (Mombelli et al.) (18)
Bleeding on probing was evaluated on four sites around each implant (mesial, distal, buccal and lingual):
Score 0: no bleeding.
Score 1: spot bleeding.
Score 2: linear bleeding.
Score 3: spontaneous bleeding.

Probing depth
Probing depth was determined by using a William's periodontal probe, mesial, distal, palatinal and buccal from the implant. All depths were noted to the nearest millimetre on the probe.

Morbidity

Complications, postoperative morbidity and patient's acceptance of the procedure were evaluated by assessing the medical records (13).

Radiographic examination

After implant placement (baseline, T0) and 12 months (T12) after loading of the implants, standardized digital intra-oral radiographs were taken using a long-cone paralleling technique as described in detail by Meijndert et al. (19) Full-screen analysis of the radiographs was performed using specifically designed software. Radiographs were calibrated according to the known diameter of the implant. Reference points for measuring peri-implant marginal bone levels were the implant/abutment junction and the first bone to implant contact. Both mesial and distal aspects of each implant were measured by one examiner (WS).

Patients' satisfaction

Patients were asked to give a score on general satisfaction about wearing the prosthetic construction before and after implant treatment. Patient's satisfaction was assessed using a rating scale ranging from 'very dissatisfied' (score 1) to 'very satisfied' (score 10) (20).

Statistics

Because of the limited number of patients, statistical analysis was restricted to descriptives.

Results

All 12 patients completed the twelve months post implant loading follow-up period. No objective and subjective sensory disturbances or complications were reported in the regions where the posterior iliac crest was punctured or were retromolar bone had been harvested.

Implants

During the osseointegration period, 3 implants (in two patients) were mobile on the test site and had to be removed (implant survival rate 91%), while no implants were mobile or had to be removed at the control site during this period (implant survival rate 100%). Because of mobility, one implant had to be removed 8 weeks and two implants had to be removed 12 weeks after implant placement. In one patient the bar-suprastructure could be made on the remaining two implants on that side, while in the other patient one of the two lost implants had to be replaced (no additional grafting was needed). After replacement healing was uneventful and also this patient could be supplied with an adequately functioning implant-supported maxillary overdenture. No implants were lost after loading of the implants.

Clinical and radiographic examination

Analysis of soft tissue parameters (*plaque-, gingiva-* and *bleeding-index, probing depth*) did not yield any difference between T0 and T12 within and between the two groups. The highest score for all indices at both times was score 1, which is clinically not relevant. Average scores for the plaque-, gingiva- and bleeding-index, were 0.12±0.37, 0.06±0.3 and 0.21±0.43, respectively. At T0 the average probing depth was 4.1±0.6mm at the test site and 4.2±1.0mm at the control site. After one year (T12) the average probing depth was 4.3±0.8mm at both sites.
Analysis of radiographic examination showed a marginal bone loss of 0.47±0.31mm and 0.41±0.25mm between T0 and T12 in the test and control group, respectively.

Patients' satisfaction

Patients' satisfaction with functioning of the maxillary denture after treatment was high. The patients rated their conventional maxillary denture as 4.5±1.6. This score improved when wearing an implant-supported maxillary overdenture (8.4±1.4).

Discussion

This study shows comparable one year post functional loading results for the two methods (BioOss® + MSCs and BioOss® + autogenous bone) applied for maxillary sinus floor eleva-

tion as described by Rickert et al. (10) for implant insertion in the posterior maxilla with respect to the peri-implant soft tissue parameters and peri-implant bone height. However, implant survival rate, a primary outcome parameter, tended to be lower in the test group. In the current study the survival rate of the test group was 91% and of the control group 100%. The survival rate of the test group is slightly lower than the range of survival rates reported in the studies reviewed by Slot et al. (2) The latter authors mentioned that the survival rate for maxillary implants placed after sinus floor elevation ranged from 94% to 100% in the first year after functional loading. This implant survival rate is also in line with the results from the systematic review by Emmerich et al. (21) reporting a short term survival rate of osteotome installed implants in partially edentulous posterior maxillary areas of 95.7% as well as other studies reporting on lower implant survival rate in maxillae between edentulous and partially edentulous patients (22-24). Finally, Visser et al. (1) reported that more favourable implant survival rates were seen in edentulous maxillae which were less resorbed which is in line with the lower implant survival rate observed in our current study on severely resorbed edentulous maxillae.

A healing period of three months is besides reducing morbidity an advantage in this study. However, cases with relatively soft bone at the time of implant placement might benefit from a longer healing period as no problems were encountered when lost implants were replaced at a later stage (the bone was probably more firm at that time). This observation and also the lower implant survival rate of the test group might indicate that a healing time of three months is at the low end for the test method, and may indicate to postpone implant placement. Yildirim et al. (12) examined sinus floor elevation procedures with only BioOss® concerning a healing time of 6.8 months. In the study of Rickert et al. (10), all implants could be placed with primary stability and 3.8 months after treatment both the test and control sites showed more new bone formation at that time point than other studies after a healing period of six up to nine months in which patients were treated with BioOss® alone (6, 25). Histomorphometrical evaluation did not show a dissimilar histomorphology between bone biopsies obtained from sites where implants were a success or where implants failed (10).

The amount of peri-implant bone loss as measured on intra-oral radiographs should be considered within the limits related to the radiographic technique. Intra-oral radiographs transform a 3D reality into a 2D projection. For this reason, some information could be missed. Analysis of radiographic examination showed slight changes in marginal bone levels. In the first year after wearing an overdenture the vertical bone loss (implant/abutment junction to the first bone/implant contact) was on average 0.47mm, respectively 0.41mm, which is clinically acceptable. These results of marginal bone loss are in accordance with previous studies, which mentioned changes of marginal bone levels varying between 0.3mm and 0.92mm (2, 26, 27).

As expected from other studies, satisfaction of the patients with regard to their wearing their implant-supported maxillary overdenture was high (1, 23, 28). Satisfaction of patients who lost implants was not lower than patients who did not lose any implants.

We admit that the sample size is small, and that the study is underpowered. Also, we are fully aware that a common way to reduce random error in, or increase precision of, an epidemiologic estimate is to enlarge the size of the study. Yet a focal problem in planning the study size is determining how to balance the value of greater precision in study results against the greater costs. Solving the problem thus involves a cost-benefit analysis of expending greater effort or funds to gain greater precision (29, 30). Greater precision has a value to the beneficiaries of the research, but the value is indeterminate because it is always uncertain how many beneficiaries there will be. If the study had more patients, theoretically it is possible that the results could change since the next "set" of patients could have a different response and thus outcome. If so, the total number of participants needed to demonstrate an effect becomes so large that the net effect is too small to be clinically important. This was reason that we performed a test of principle study to elucidate whether survival of implants is reasonable and condition of peri-implant tissues is similar to that of conventional approaches. For such study, a number of 12 patients suffices, particularly when the patient is his own control, to answer the treatment related condition of peri-implant tissues in particular.

In conclusion, the two techniques (BioOss® + MSCs and BioOss® + autogenous bone) for maxillary sinus floor elevation as a reconstructive procedure to enable implant insertion in the posterior maxilla at a later stage as described by Rickert et al. (11) were shown to be equally reliable methods regarding peri-implant soft tissue parameters and reducing donor site morbidity. However, it is questionable, if the lower implant survival rate observed in the test group is a real issue or is mainly due to the number of implants placed which makes that larger differences between groups may occur rather easily. To judge whether the approach applying a posterior iliac crest bone marrow concentrate enriched in MSCs is a valid clinical asset to reduce donor site morbidity, a slightly longer healing time has to be considered. This issue has to be assessed in future studies. When taking the lower implant survival rate into account, it is not yet recommended to use a bone marrow concentrated enriched in MSCs as an alternative to autogenous bone when a healing period of three to four months, as commonly accepted for the sole used of autogenous bone, is regarded before implant placement.

References

1. Visser A, Raghoebar GM, Meijer HJ, Vissink A, Implant-retained maxillary overdentures on milled bar suprastructures: a 10-year follow-up of surgical and prosthetic care and aftercare. Int J Prosthodont 2009: 22:181-192.

2. Slot W, Raghoebar GM, Vissink A, Huddleston Slater JJ, Meijer HJ A systematic review of implant-supported maxillary overdentures after a mean observation period of at least 1 year. J Clin Periodontol 2010: 37: 98-110.

3. Ulm CW, Solar P, Gsellmann B, Matejka M, Watzek G, The edentulous maxillary alveolar process in the region of the maxillary sinus--a study of physical dimension. Int J Oral Maxillofac Surg 24 1995: 279-282.

4. Jensen OT, Shulman LB, Block MS, Iacono VJ, Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 2010: 13:11-45.

5. Rickert D, Huddleston Slater JJR, Meijer HJA, Vissink A, Raghoebar GM, Maxillary sinus lift with solely autogenous bone compared to a combination of autogenous bone and growth factors or (solely) bone substitutes. A systematic review. Int J Oral Maxillofac Surg 2012: 41: 160-116.

6. Hallman M, Sennerby L, Lundgren S, A clinical and histologic evaluation of implant integration in the posterior maxilla after sinus floor augmentation with autogenous bone, bovine hydroxyapatite, or a 20:80 mixture. Int J Oral Maxillofac Implants 2002: 17:635-643.

7. Nkenke E, Stelzle F, Clinical outcomes of sinus floor augmentation for implant placement using autogenous bone or bone substitutes: a systematic review. Clin Oral Implants Res 2009: 20:124-133.

8. Jensen T, Schou S, Stavropoulos A, Terheyden H, Holmstrup P, Maxillary sinus floor augmentation with Bio-Oss or Bio-Oss mixed with autogenous bone as graft in animals: a systematic review. Int J Oral Maxillofac Surg 2009: 41:114-120.

9. Gutwald R, Haberstroh J, Kuschnierz J, Kister C, Lysek DA, Maglione M, Xavier SP, Oshima T, Schmelzeisen R, Sauerbier S, Mesenchymal stem cells and inorganic bovine bone mineral in sinus augmentation: Comparison with augmentation by autologous bone in adult sheep. Br J Oral Maxillofac Surg 2010: 48:285-290.

10. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoebar GM, Maxillary sinus floor elevation with Bio-Oss® combined with either autogenous bone or autogenous stem cells. A prospective randomized clinical trial. Clin Oral Implants 2011: Res 22:251-258.

11. Sauerbier S, Rickert D, Gutwald R, Nagursky H, Oshima T., Xavier SP, Christmann J, Kurz P, Menne D, Vissink A, Raghoebar G, Schmelzeisen R, Wagner W, Koch FP, Bone marrow concentrate and bovine bone mineral for sinus floor augmentation: a controlled, randomized, single-blinded clinical and histological trial-- per-protocol analysis. Tissue Eng Part A 2011: 17: 2187-2197.

12. Yildirim M, Spiekermann H, Biesterfeld S, Edelhoff D, Maxillary sinus augmentation using xenogenic bone substitute material Bio-Oss in combination with venous blood. A histologic and histomorphometric study in humans. Clin Oral Implants Res 2000: 11: 217-229.

13. Raghoebar GM, Meijndert L, Kalk WW, Vissink A, Morbidity of mandibular bone harvesting: a comparative study. Int J Oral Maxillofac Implants 2000: 22: 359-365.

14. Zerbo IR, Zijderveld SA, de Boer A, Bronckers AL, de Lange G, ten Bruggenkate CM, Burger EH Histomorphometry of human sinus floor augmentation using a porous beta-tricalcium phosphate: a prospective study. Clin Oral Implants Res 2004: 15: 724-732.

15. Slot W, Raghoebar GM, van Dijk G, Meijer HJA, Modified design of an attachment system for the maxillary overdenture; a clinical report. J Prosthet Dent 2004 (accepted).

16. Silness J, Loe H Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964: 22:122-135.

17. Loe H, Silness J, Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963: 21:533-551.

18. Mombelli A, van Oosten MAC, Schürch E, Lang NP. The microbiota associated with successful or failing osseointegrated titanium implants. Oral Microbiol Immunol 1987: 2:145-151.

19. Meijndert L, Meijer HJ, Raghoebar GM, Vissink A. A technique for standardized evaluation of soft and hard peri-implant tissues in partially edentulous patients. J Periodontol 2004: 75: 646-651.

20. Meijer HJ, Raghoebar GM, Van 't Hof MA. Comparison of implant-retained mandibular overdentures and conventional complete dentures: a 10-year prospective study of clinical aspects and patient satisfaction. Int J Oral Maxillofac Implants 2003: 18: 879-885.

21. Emmerich D, Att W, Stappert C.Sinus floor elevation using osteotomes: a systematic review and meta-analysis. J Periodontol 2005: 76: 1237–1251.

22. Esposito M, Hirsch JM, Lekholm U, Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. 1. Success criteria and epidemiology. Eur J Oral Sci 1998a: 106: 527–551.

23. Raghoebar GM, Timmenga NM, Reintsema H, Stegenga B, Vissink A. Maxillary bone grafting for insertion of endosseous implants: results after 12-124 months. Clin Oral Implants Res 2001: 12:279-286.

24. Lang NP, Pjetursson BE, Tan K, Brägger U, Egger M, Zwahlen M. A systematic review of the survival and complication rates of fixed partial dentures (FPDs) after an observation period of at least 5 years. II. Combined tooth--implant-supported FPDs. Clin Oral Implants Res 2004: 15: 643-653.

25. Froum SJ, Wallace SS, Elian N, Cho SC, Tarnow DP. Comparison of mineralized cancellous bone allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: histomorphometry at 26 to 32 weeks after grafting. Int J Periodontics Restorative Dent 2006: 26: 543-551.

26. Zijderveld SA, Schulten EA, Aartman IH, ten Bruggenkate CM. Long-term changes in graft height after maxillary sinus floor elevation with different grafting materials: radiographic evaluation with a minimum follow-up of 4.5 years. Clin Oral Implants Res 2009: 20:691-700.

27. Cricchio G, Sennerby L, Lundgren S. Sinus bone formation and implant survival after sinus membrane elevation and implant placement: a 1- to 6-year follow-up study. Clin Oral Implants Res 2009: 22: 1200-1212.

28. Pjetursson BE, Karoussis I, Bürgin W, Brägger U, Lang NP. Patients' satisfaction following implant therapy. A 10-year prospective cohort study. Clin Oral Implants Res 2009: 16:185-193.

29. Robinson R. Cost-effectiveness analysis. BMJ 1993: 25:793-795.

30. Langley PC. Cost effectiveness and the allocation of therapies in a treating population. Pharmacoeconomics 1996: 10:93-98.

Chapter 7

General discussion

General discussion

Implant procedures in the posterior maxilla often pose a problem for edentulous patients with an atrophic maxilla due to an insufficient bone volume. This restriction is not reserved to edentulous patients, but also is often observed in partial dentate patients needing an implant-based prosthodontic rehabilitation in the posterior region of the maxilla. An insufficient volume of bone to allow reliable primary placement of implants in this region is usually solved by a maxillary sinus floor elevation procedure. This PhD study was aimed to evaluate different approaches aiming reduction of the morbidity accompanying sinus elevation surgery.

From the systematic review of the literature (**chapter 2**), evaluating studies in which the bone volume after sinus floor elevation surgery was evaluated by histomorphological analysis, it is obvious that adequate bone formation in a created space (e.g. the space created between the residual maxillary ridge and the elevated Schneiderian membrane) can be achieved with a variety of materials when a reasonable healing period (3-6 months depending on the material used has been allowed. For sinus floor augmentations, autogenous bone is the most common used material and is still considered the gold standard (1-3), although numerous alternative materials have been used with variable results.
Bone substitutes, such as bovine hydroxyapatite, bioactive glass or corticocancellous pig bone in combination with autogenous bone were shown to provide a reliable alternative for autogenous bone as a sole grafting material to reconstruct bony deficiencies in the maxillary sinus region, for supporting dental implants when allowing for an at least five months bone healing time. With regard to bioactive glass particles, when combined with autogenous bone, this substitute is also an alternative to the sole use of autogenous bone when a healing period of at least 5 months is considered (5). Addition of growth factors (platelet rich plasma) to a grafting material as well as the sole use of β-tricalciumphosphate did not promote the formation of new bone (7-9). Thus, bone substitutes are a proper alternative to autogenous bone when aiming for a bone volume sufficient to place implants. A major advantage of the use of bone substitutes is a reduction of the morbidity as no or a lesser amount of autogenous bone has to be harvested, but a major disadvantage is the much longer healing time that is needed before implants can be installed. This healing time is at least double the minimum needed when compared to the sole use of autogenous bone. Therefore, in this PhD research new methods were assessed to reduce the morbidity of the grafting procedure as well as to enable placement of implants within a time frame comparable to that of the sole use of autogenous bone.

First, it was assessed whether the morbidity related to a perforation of the Scheiderian membrane, which occurs in most instances either while using rotative instruments to make the window or when using hand instruments to gain initial access to begin the elevation of the membrane from the sinus walls, could be reduced by using ultrasonic waves for bone

cutting instead of rotative instruments (**chapter 3**). An important achievement of the use of ultrasonic waves, using a piezoelectric device, is the much lower risk of causing visible injury to the adjacent soft tissues (15-17). It was shown that piezoelectric bone surgery was a reliable alternative to the use of conventional rotative instruments as the results of both techniques were comparable. This observation is in agreement with the clinical results reported by Barone and collegues (18). The only limitation of piezosurgery observed in our study was the time factor as the operation time was significantly shorter when using conventional rotative instruments. This observation is in agreement with the studies of Barone and colleagues, Kotrikova and colleagues and Landes and colleagues (18-20), but the difference in operation time between both operative procedures for maxillary bone is, from a clinical perspective, negligible. Therefore, it can be concluded that both techniques can be used in clinical practice, depending on the preference of the operator. It is the experience of the surgeon in using conventional rotative instruments instead of piezoelectric surgery that is leading whether perforations will occur and what operation time is needed. That also means that piezoelectric surgery only is reliable if the surgeon does have sufficient experience in using piezoelectric surgery and reverse for rotative instruments.

Next, it was studied whether the incorporation of bovine bone (Bio-Oss®) as the only grafting material used to reconstruct a maxillary defect could be enhanced to allow for earlier implant placement when compared to the combined use of Bio-Oss® with autogenous bone. The use of bovine bone in combination with autogenous bone offers some advantages. First, it allows the volume of the graft to be at least doubled, avoiding the need to harvest large amounts of autogenous bone. Second, the osteoconductive properties of bovine bone act as a scaffold that is essential for bone remodeling. Third, bovine bone is a calcium-deficient carbonate apatite with a crystal size of approximately 10 nm. Therefore, the surface area of each graft particle is considerably greater than that of porous bioceramics, making its resorption considerably slower (10). A major disadvantage of the use of bovine bone mixed with autogenous bone is the need for a donor site with its inherent morbidity.
Therefore, it was assessed whether the procedure of adding autogenous bone marrow fraction enriched in mesenchymal stem cells (MSCs) to biomaterials as BioOss® could be modified in such a way that donor site morbidity can be reduced and the healing time is not longer than when applying autogenous bone as the sole grafting material. As shown in **chapters 4-6** replacement of autogenous bone by a bone marrow fraction enriched in mesenchymal stem cells (MSCs) resulted in a comparable level of formation of new bone after a healing time of 3-4 months when compared to the sole use of autogenous bone. The observed formation of new bone was comparable to that observed when applying bovine bone as a sole grafting material when allowing for a healing time of at least 6 to 8 months (22). Also implant survival in areas reconstructed with a mixture of bovine bone and MSCs was almost comparable to that of areas reconstructed with only autogenous bone, although with a slight tendency of increased implant loss early after grafting.
The primary endpoint of these trials was percentage of new bone three months after the el-

evation procedure. This technique seemed to be an alternative to auto-grafts, however also evaluation was needed with respect to implant placement and functional loading. Therefore we analyzed performance of this technique in a one year follow-up study (**chapter 6**). After one year in function, no clinically relevant differences were observed regarding soft tissue parameters and peri-implant bone loss. However, implant survival rate tended to be lower in the test group. To judge whether the approach applying a posterior iliac crest bone marrow concentrate enriched in MSCs is a valid clinical asset to reduce donor site morbidity, a slightly longer healing time has to be considered. This issue has to be assessed in future studies. When taking the lower implant survival rate into account, it is not yet recommended to use a bone marrow concentrate enriched in MSCs as an alternative to autogenous bone with a healing period of three to four months, as commonly accepted for the sole use of autogenous bone.

Notwithstanding the promising results of the above discussed method, the need for a grafting material to fill the space between the maxillary crest and a lifted Scheiderian membrane may become questionable as recent studies have demonstrated that the mere lifting of the sinus mucosal lining and simultaneous placement of implants also can result in bone formation (40, 41). However, currently this technique only is applied for conditions allowing for sufficient primary stability of implants during placement and a sufficient width of the alveolar crest.

General conclusions and future perspectives

In this PhD study different approaches aiming for reduction of the morbidity accompanying sinus elevation surgery were evaluated. From the systematic review of the literature it was obvious that short-term implant survival was not dependent on grafting procedures applied when allowing for a sufficient healing time before implant placement (**chapter 2**). With regarding to use of a piezo electric device as an alternative for the use of conventional rotative instruments in sinus membrane elevation surgery, it was shown that the results of both approaches were comparable (**chapter 3**), so the surgeon should select that techniques that suites him the best. Finally, from our MSCs studies it was obvious that the bovine bone + MSCs grafting approach seems to be a proper alternative to the sole use of autogenous bone when aiming for a procedure with a comparable healing time but less donor site morbidity (**chapters 4-6**).

The results of this study have been translated to hypotheses as how to perform sinus floor elevation surgery when aiming for a combination of minimal donor site morbidity and a short healing time. With regard to the results for this technique, trials with different study protocols are needed before approval can be obtained. First a longer follow-up is needed to assess long term results. Additional attention should be paid on studies in which a longer healing time is taken into account to increase implant survival rate. However, this will mean that one advantage in our study decreasing the healing period, is disappeared. Furthermore, a larger number of patients have to be studied based on a power analysis using the results of our study as input data.

References

1. Hallman M & Thor A. Bone substitutes and growth factors as an alternative/complement to autogenous bone for grafting in implant dentistry. Periodontol 2000 2008: 47: 172-192.

2. Jensen OT, Shulman LB, Block MS, Iacono VJ. Report of the Sinus Consensus Conference of 1996. Int J Oral Maxillofac Implants 1998: 13: 11-45.

3. Tong DC, Rioux K, Drangsholt M, Beirne OR. A review of survival rates for implants placed in grafted maxillary sinuses using meta-analysis. Int J Oral Maxillofac Implants 1998: 13: 175-182.

4. Tadjoedin ES, de Lange GL, Holzmann PJ, Kulper L, Burger EH. Histological observations on biopsies harvested following sinus floor elevation using a bioactive glass material of narrow size range. Clin Oral Implants Res 2000: 1: 334-344.

5. Bettega G, Brun JP, Boutonnat J, Cracowski JL, Quesada JL, Hegelhofer H, Drillat P, Richard MJ. Autologous platelet concentrates for bone graft enhancement in sinus lift procedure. Transfusion 2009: 49: 779-785.

6. Raghoebar GM, Schortinghuis J, Liem RS, Ruben JL, van der Wal JE, Vissink A. Does platelet-rich plasma promote remodeling of autologous bone grafts used for augmentation of the maxillary sinus floor? Clin Oral Implants Res 2005: 16: 349-356.

7. Schaaf H, Streckbein P, Lendeckel S, Heidinger K, Gortz, B, Bein G, Boedeker RH, Schlegel KA, Howaldt HP. Topical use of platelet-rich plasma to influence bone volume in maxillary augmentation: a prospective randomized trial. Vox Sang 2008: 94: 64-69.

8. Vercellotti T.. Technological characteristics and clinical indications of piezoelectric bone surgery. Minerva Stomatol 2004: 53: 207-214.

9. Eggers G, Klein J, Blank J, Hassfeld S. Piezosurgery: an ultrasound device for cutting bone and its use and limitations in maxillofacial surgery. Br J Oral Maxillofac Surg 2004: 42: 451-453.

10. Wallace SS, Mazor Z, Froum SJ, Cho SC, Tarnow DP. Schneiderian membrane perforation rate during sinus elevation using piezosurgery: clinical results of 100 consecutive cases. Int J Periodontics Restorative Dent 2007: 27: 413-419.

11. Barone A, Santini S, Marconcini S, Giacomelli L, Gherlone E, Covani U. Osteotomy and membrane elevation during the maxillary sinus augmentation procedure. A comparative study: piezoelectric device vs. conventional rotative instruments. Clin Oral Implants Res 2008: 19: 511-515.

12. Kotrikova B, Wirtz R, Krempien R, Blank J, Eggers G, Samiotis A, et al. Piezosurgery--a new safe technique in cranial osteoplasty? Int J Oral Maxillofac Surg 2006: 35: 461-465.

13. Landes CA, Stubinger S, Rieger J, Williger B, Ha TK, Sader R. Critical evaluation of piezoelectric osteotomy in orthognathic surgery: operative technique, blood loss, time requirement, nerve and vessel integrity. J Oral Maxillofac Surg 2008: 66: 657-674.

14. Galindo-Moreno P, Avila G, Fernandez-Barbero JE, Aguilar M, Sanchez-Fernandez E, Cutando A, Wang HL. Evaluation of sinus floor elevation using a composite bone graft mixture. Clin Oral Implants Res 2007: 18: 376-382.

15. Froum SJ, Wallace SS, Elian N, Cho SC, Tarnow DP. Comparison of mineralized cancellous bone allograft (Puros) and anorganic bovine bone matrix (Bio-Oss) for sinus augmentation: histomorphometry at 26 to 32 weeks after grafting. Int J Periodontics Restorative Dent 2006: 26: 543-551.

16. Lundgren S, Cricchio G, Palma VC, Salata LA, Sennerby L. Sinus membrane elevation and simultaneous insertion of dental implants: a new surgical technique in maxillary sinus floor augmentation. Periodontol 2000 2008: 47: 193-205.

17. Raghoebar GM, Meijer HJ, Telleman G, Vissink A. Maxillary Sinus Floor Augmentation Surgery with Autogenous Bone Grafts as Ceiling: A Pilot Study and Test of Principle. Clin Implant Dent Relat Res 2011 Dec 15. [Epub ahead of print]

Summary

Summary

Implant placement in the posterior maxilla is often compromised by a lack of bone volume to allow for reliable implant placement. This restriction is not reserved to edentulous patients with an atrophic maxilla, but is often observed in partial dentate patients needing an implant-based prosthodontic rehabilitation in this region. To provide a sufficient bone volume for implant placement in this region usually a maxillary sinus floor elevation procedure is performed. This PhD study aimed to evaluate different approaches aiming for reduction of the morbidity accompanying sinus floor elevation surgery.

In the first part of this thesis, a systematic review of the literature is described. This review focussed on the treatment outcome of residual maxillary ridges needing maxillary sinus floor elevation surgery to create a bone volume sufficient to allow for reliable implant placement in human. The objectives of this review were to assess the bone fraction and implant survival rate in the reconstructed posterior maxillae and to determine whether the bone fraction was affected by the grafting material or growth factor applied (**Chapter 2**). MEDLINE (1979– September 2010, via PUBMED) and EMBASE (1987- September 2010) were explored for trials in which sinus floor elevations with autogenous bone (control group) were compared with autogenous bone in combination with growth factors or bone substitutes, or solely with bone substitutes (test groups). Histomorphometric analysis was mandatory to compare results properly. Twelve out of 1124 selected studies fulfilled all inclusion criteria. Meta-analyses comparing the bone fraction after applying autogenous bone, a combination of autologous bone with growth factors (platelet rich plasma), or a combination of autogenous bone and bone substitutes (bovine hydroxyapatite, bioactive glass, corticocancellous pig bone) revealed no significant differences in formation of new bone after a healing period of at least 5 months ($p=0.341$, $p=0.821$, $p=0.372$, $p=0.609$, respectively, for bovine hydroxyapatite, bioactive glass and corticocancellous pig bone), while a significantly higher bone fraction in the autogenous bone group was observed when compared to the sole use of β-tricalciumphosphate ($p=0.036$). The one-year overall implant survival rate showed no significant difference between implants placed in control or test sites (97.2% versus 98.2%, respectively).
From this study it was concluded that bone substitutes, such as bovine hydroxyapatite, bioactive glass or corticocancellous pig bone in combination with autogenous bone provide a reliable alternative for autogenous bone as a sole grafting material to reconstruct bony deficiencies in the maxillary sinus region, for supporting dental implants when allowing for an at least five months bone healing time. Addition of growth factors (platelet rich plasma) to a grafting material as well as the sole use of β-tricalciumphosphate did not promote the formation of new bone.

In the second part of this thesis, the focus was on reducing the morbidity of the approach used to assess the lateral wall of the maxillary sinus and to elevate the Schneiderian mem-

brane. To comply with the quest for minimally invasive surgery, ultrasonic waves have been introduced for bone cutting in oral and maxillofacial surgery. An important achievement of this approach, using a piezoelectric device, is the much lower risk on causing visible injury to the adjacent soft tissues. The piezoelectric device has been reported to decrease the risk of damage to surrounding soft tissues and many other critical structures (nerves, vessels). Therefore, to compare the use of conventional rotative instruments and a piezoelectric device for maxillary sinus floor elevation surgery, a randomized clinical trial was designed (**Chapter 3**). Thirty-six consecutive patients (59.2±10.7 years, range 38-76 years) needing bilateral sinus floor elevation surgery agreed to participate in this parallel split mouth designed trial. The allocation of the surgical technique to be used on the determined sites was randomly assigned, one site was always treated with conventional rotative instruments (control group) and the other site with piezosurgery (test group). In addition, in a random order, the grafted sites were covered with a collagen membrane or no membrane. After a healing period of 3-4 months implants were placed. Comparison of clinical features of the test and control sites revealed no differences with regard to wound healing and complications (perforations of the sinus membrane) during or post surgery ($p=0.458$, $p=1.0$, respectively). A clinically insignificant, but statistically shorter operation time was observed when using conventional rotative instruments (11.1±2.4 min) than using piezosurgery (15.1±2.9 min; $p<0.001$). In both groups, application of a resorbable membrane did not result in less horizontal bone resorption (membrane: 1.43 mm, no membrane: 1.06 mm; $p=0.062$). All 193 implants could be placed with primary stability. One year after functional loading survival rate was 100%.
From this study it can be concluded that for maxillary sinus floor elevation surgery piezoelectric device forms a reliable alternative to the use of conventional rotative instruments.

In the third part of this thesis, the focus was on the clinical evaluation of a recently introduced method to reduce the morbidity of iliac crest bone harvesting and to promote formation of new bone in sites reconstructed with bovine bone (BioOss®). For this method, bone marrow is aspirated from the posterior iliac crest. Next, the bone marrow is treated in such a way that a fraction is obtained rich in mesenchymal stem cells (MCSs). This concentrate is added to the BioOss® that is used as a bone substitute. The aim of the two randomized controlled trials described in **chapters 4** and **5** was to assess whether differences in bone formation occur after maxillary sinus floor elevation surgery with either autogenous bone in combination with BioOss® or BioOss® seeded with a bone marrow concentrate from posterior iliac crest rich in MSCs. The primary endpoint was percentage of new bone three months after the elevation procedure. In **chapter 6**, the effect of this new approach on one year implant survival and peri-implant tissues was assessed.
In the study described in **chapter 4**, 45 severely atrophied maxillary sinus from 26 patients were evaluated in a partial cross-over design. As test arm, 34 sinus of 25 patients were augmented with bovine bone mineral (BioOss®) and bone marrow aspirate concentrate containing MSCs. Eleven control sinus from 11 patients were augmented with a mixture of

70% BioOss® and 30% autogenous bone. Biopsies were obtained after 3-4 months healing period at time of implant placement and histomorphometrically analysed for new bone formation. New bone formation was 14.3±1.8% for the controls and non-significantly lower at 12.6±1.7% for the test sites.

In the study described in **chapter 5**, in a randomized controlled split-mouth design in 12 consecutive patients (age 60.8±5.9 years, range 48-69 years) needing reconstruction of their atrophic maxilla, a bilateral sinus floor augmentation procedure was performed. Randomly, on one site the augmentation procedure was performed with BioOss® seeded with bone marrow fraction enriched in mononuclear stem cells harvested from the posterior iliac crest (test group) while BioOss® mixed with autogenous bone (harvested from the retromolar area) was applied on the contra-lateral site (control group). On 14.8±0.7 weeks after the sinus floor elevation, biopsies from the reconstructed areas were taken at the spots were subsequently the endosseous implants were placed (in total 66 Straumann Standard Implants®). The biopsies were histomorphometrically analyzed. Significantly more bone formation was observed in the test group (17.7%±7.3) when compared to the control group (12.0%±6.6; p=0.026). In both the test and control group, all implants could be placed with primary stability. In one patient not all biopsies contained BioOss®. This patient was excluded from analysis.

From the studies described in **chapter 4** and **5** it can be concluded that new bone formation after 3-4 months is equivalent in sinus, augmented with bone marrow aspirate concentrate and bovine bone mineral or a mixture of autogenous bone and BioOss®. This technique could be an alternative to using autografts to stimulate bone formation.

Next, in **chapter 6**, to assess implant survival and one-year clinical performance regarding implants placed in the posterior maxilla that had been subjected to maxillary sinus floor elevation surgery with BioOss® mixed with an autogenous bone marrow concentrate or autogenous bone a follow-up study was performed in the patients of the study described in chapter 5. At baseline and twelve months after functional loading, implant survival, plaque-, gingival-, bleeding-indices, probing depth and peri-implant radiographic bone levels were assessed. During osseointegration, three implants failed at the test site (2 patients), no implants failed at the control site, resulting in three months survival rates of 91% and 100%. No implants were lost after functional loading. Neither differences in soft tissue parameters nor in peri-implant bone loss were observed between control and test sites during follow up. After one year in function, no clinical relevant differences were observed regarding soft tissue parameters and peri-implant bone loss. However, implant survival rate tended to be lower in the test group.

In the last part of this PhD thesis, the main research outcomes are discussed and general conclusions are drawn (**chapter 7**). From the systematic review of the literature it was obvious that short-term implant survival was not dependent on grafting procedures applied when allowing for a sufficient healing time before implant placement (**chapter 2**). With regard to use of a piezo electric device as an alternative for the use of conventional rota-

tive instruments in sinus membrane elevation surgery, it was shown that the results of both approaches were comparable (**chapter 3**), so the surgeon should select that techniques that suites him the best. Finally, from our MSCs studies it was obvious that the bovine bone + MSCs grafting approach seems to be a proper alternative to the sole use of autogenous bone when aiming for a procedure with a comparable healing time but less donor site morbidity (**chapters 4** and **5**). One year implant survival in test sites was lower, but condition of peri-implant tissues was comparable between control and test sites (**chapter 6**). The results of this study have been translated to hypotheses as how to perform sinus floor elevation surgery when aiming for a combination of minimal donor site morbidity and a short healing time. These hypotheses have to be tested in future studies.

Samenvatting

Samenvatting

Na het edentaat worden van de bovenkaak, treedt naast resorptie van de processus alveolaris, vaak uitzakking van de sinus maxillaris op (pneumatisatie). Vanwege de resorptie van de processus kan het houvast van een bovenprothese afnemen. Vanwege de pneumatisatie is het vaak niet mogelijk om zonder het aanbrengen van een bottransplantaat implantaten in de zijdelingse delen van de bovenkaak te plaatsen. In dergelijke situaties biedt een botopbouw in de vorm van een sinusbodemelevatie veelal uitkomst om voldoende botvolume te creëren voor het betrouwbaar kunnen plaatsen van implantaten.
De procedure voor een sinusbodemelevatie is voor het eerst beschreven en uitgevoerd in de jaren '80 van de vorige eeuw. Bij de klassieke sinusbodemelevatie wordt een botluikje in de laterale wand van de sinus maxillaris geprepareerd welke, na het mobiliseren van de membraan van de sinus maxillaris, naar craniaal of mediaal kan worden verplaatst. De ontstane ruimte kan worden opgevuld met een autoloog bottransplantaat, een botsubstituut of een mengsel van autoloog bot met een botsubstituut. Dit proces wordt augmentatie genoemd.
Momenteel is autoloog bot, eventueel in combinatie met een botsubstituut zoals gedemineraliseerd runderbot (Bio-Oss®), de eerste behandelkeuze voor het verhogen van de bodem van de sinus maxillaris. Voor het oogsten van autoloog bot zijn verschillende extra- en intra-orale donorplaatsen mogelijk. De meest gebruikte intra-orale donorplaatsen zijn de retromolaar regio in de onderkaak, de kin en het tuber maxillare. De morbiditeit van het oogsten van bot in de mond is relatief gering en behelst vooral het optreden van pijn, bloedingen en sensibiliteitsstoornissen. Indien een grotere hoeveelheid bot benodigd is, wordt het bot veelal extra-oraal geoogst. De crista iliaca anterior is een veel gebruikte donorplaats, met als belangrijk nadeel de morbiditeit van de donorplaats. De patiënt kan gedurende een aantal weken veel hinder ondervinden bij het bewegen, in het bijzonder bij het lopen. Daarom zijn in deze PhD-studie verschillende behandelmethodes voor het ophogen van de sinus maxillaris beschreven met als doel de morbiditeit van de ingreep zo laag mogelijk en de genezingsperiode zo kort mogelijk te houden.

In **hoofdstuk 2** wordt een systematische literatuurstudie naar het behandelresultaat van een elevatie van de sinus maxillaris voor het creëren van een voldoende botvolume voor het plaatsen van implantaten in de zijdelingse delen van de bovenkaak beschreven. Vooral werd nagegaan of het uitmaakt of hiertoe autoloog bot of een botsubstituut wordt toegepast en of het uitmaakt dat autoloog bot of een botsubstituut wordt gecombineerd met groeifactoren. Hiervoor werden MEDLINE (1979– September 2010, via PUBMED) en EMBASE (1987- September 2010) doorzocht. Geïncludeerd werden studies waarin een sinusbodemelevatie met autoloog bot (controle groep) werd vergeleken met autoloog bot in combinatie met een botsubstituut, met alleen een botsubstituut, of met autoloog bot of een botsubstituut gecombineerd met groeifactoren (test groepen). De methodologische kwaliteit van deze studies werd beoordeeld door twee beoordelaars, onafhankelijk van elkaar en aan de hand van vaste criteria.

Van de 1124 geselecteerde studies, bleken 12 artikelen te voldoen aan de vooraf opgestelde in- en exclusiecriteria. Een meta-analyse van de geïncludeerde studies liet geen significante verschillen in botnieuwvorming zien tussen autoloog bot en autoloog bot in combinatie met groeifactoren (platelet rich plasma), of tussen autoloog bot en autoloog bot gecombineerd met een botsubstituut (zoals gedemineraliseerd runderbot, bioactief glas en varkensbot) bij een genezingsperiode van ten minste 5 maanden. Echter t.o.v. het gebruik van alleen een botsubstituut (β-tricalciumphosphate) bleek de botnieuwvorming in de test groep veel langzamer te verlopen dan in de controle groep. De overlevingspercentages van de implantaten waren onafhankelijk van het toegepaste autologe bot of botsubstituut. Op basis van de resultaten van deze studie kan worden geconcludeerd dat botsubstituten zoals gedemineraliseerd runderbot, bioactief glas of varkensbot in combinatie met autoloog bot, een alternatief bieden voor het gebruik van alleen autoloog bot als reconstructiemateriaal. Wanneer gebruik wordt gemaakt van alleen een botsubstituut moet een langere genezingstijd (ten minste 5 maanden) in acht worden genomen. Het toevoegen van groeifactoren aan het reconstructiemateriaal bleek niet te leiden tot meer botnieuwvorming.

Er zijn vele chirurgische technieken beschreven voor sinusbodemelevatie chirurgie. De meest voorkomende intra-operatieve complicatie van al deze technieken is het perforeren van de membraan van Schneider, de membraan die de sinus maxillaris bekleed. Een perforatie van deze membraan kan o.a. worden veroorzaakt tijdens de preparatie van het "botluikje" met roterend instrumentarium. De kans op dergelijke perforaties kan mogelijk worden verkleind door gebruik te maken van Piezochirurgie. Om na te gaan of het gebruik van Piezochirurgie inderdaad tot minder complicaties leidt dan het gebruik van conventioneel roterend instrumentarium werd een gerandomiseerde klinische studie uitgevoerd waarbij conventioneel roterend instrumentarium werd vergeleken met Piezochirurgie (**hoofdstuk 3**). In totaal werd bij 36 patiënten een bilaterale sinusbodemelevatie uitgevoerd. Na randomisatie werd aan de ene zijde de sinusbodemelevatie uitgevoerd met de conventionele techniek (controle groep), terwijl aan de contralaterale zijde de sinusbodemelevatie werd uitgevoerd d.m.v. Piezochirurgie (test groep), een zogenaamd 'split mouth' design. Bovendien werd, ook na randomisatie, wel of geen collageenmembraan over het aangebrachte bottransplantaat aangebracht. Na een genezingsperiode van 3-4 maanden werden implantaten geplaatst. Tijdens of na de ingreep werden klinisch geen significante verschillen gezien tussen beide groepen met betrekking tot wondgenezing en complicaties zoals het perforeren van de membraan van Schneider. Klinisch minder relevant, maar wel significant, was de kortere operatietijd bij het gebruik van conventioneel roterend instrumentarium (11.1±2.4 min) dan bij het gebruik van Piezochirurgie (15.1±2.9 min; $p<0.001$). De mate van horizontaal botverlies bleek onafhankelijk te zijn van het wel of niet aanbrengen van een collageenmembraan . Alle 193 implantaten konden met voldoende primaire stabiliteit worden geplaatst. Een jaar na belasting was het implantaatoverlevingspercentage 100%. Op grond van deze studie werd geconcludeerd dat Piezochirurgie een alternatief is voor het gebruik van conventioneel roterend instrumentarium, maar hier niet superieur noch inferieur aan is.

Zoals al eerder genoemd, is de morbiditeit na het oogsten van intra-oraal bot relatief gering. Is echter een grotere hoeveelheid bot benodigd, dan wordt het bottransplantaat vaak uit de crista iliaca anterior geoogst, hetgeen met een grotere morbiditeit gepaard gaat. Om de morbiditeit van de procedure voor het reconstrueren van grote(re) botdefecten van de zijdelings delen van de bovenkaak te verminderen en tegelijkertijd wel de voordelen van autologe materialen te benutten, werd bestudeerd of een in proefdieren ontwikkelde minder invasieve methode ook klinisch tot de gewenste resultaten leidt (**hoofdstukken 4** en **5**). Via een punctie van de crista iliaca posterior werd 50 ml bloed afgenomen, conform de procedure voor een beenmergpunctie. Het aspiraat werd via een aantal centrifugestappen zodanig bewerkt dat een met meschenchymale stamcellen (MSCs) verrijkte fractie werd verkregen. Deze met MSCs verrijkte fractie werd gemengd met runderbot (BioOss®).
In **hoofdstuk 4** werden 45 sinusbodemelevaties bij 26 patiënten geëvalueerd. In de test groep werden 34 sinussen van 25 patiënten geaugmenteerd met BioOss® gemengd met MSCs. In de controle groep werden 11 sinussen van 11 patiënten (waarvan 10 split mouth) behandeld met een mengsel van 70% BioOss® en 30% autoloog bot. Na 3-4 maanden genezing werden tijdens het plaatsen van implantaten biopten genomen. Deze botbiopten werden histomorphometrisch geanalyseerd. Hierbij werd gekeken naar botnieuwvorming. In de controle groep werd de vorming van 14.3±1.8% nieuw bot gezien, in de controle groep lag dit iets, maar niet significant, lager (12.6±1.7%).
In een subgroep van de in **hoofdstuk 4** beschreven studie, werd een bilaterale sinusbodemelevatie uitgevoerd (12 patiënten). In een zogenaamde 'split mouth' opzet werd ad random aan een zijde de augmentatie verricht met een combinatie van BioOss® en een beenmergconcentraat rijk aan MSCs, en aan de controle zijde met een mengsel van een intra-oraal gewonnen autoloog bottransplantaat (70%) en Bio-Oss® (30%) (**hoofdstuk 5**). 14.8±0.7 weken na de elevatieprocedure werd in de test groep significant meer nieuwe botvorming (17.7%±7.3) gezien dan in de controle groep (12.0%±6.6; p=0.026).
Uit de in de **hoofdstukken 4** en **5** beschreven studies werd geconcludeerd dat de combinatie van BioOss® met toevoeging van een beenmergconcentraat rijk aan MSCs een goed alternatief lijkt te zijn voor reconstructie van de maxilla ten behoeve van het plaatsen van implantaten. De in beide hoofdstukken beschreven resultaten zijn echter gebaseerd op histologisch onderzoek. Of de resultaten ook klinisch goed blijken te zijn, werd één jaar na het functioneel belasten van de implantaten beoordeeld in de groep van 12 patiënten die een bilaterale augmentatie van de bodem van de sinus maxillaris hadden ondergaan (**hoofdstuk 6**). Bij deze patiënten waren drie tot vier maanden na de elevatieprocedure in totaal 66 Straumann Standard Implants® geplaatst. De plaque-, gingiva-, en bloedingindex, de pocketdiepte en het peri-implantaire botniveau werd voorafgaand en één jaar na het functioneel belasten van de implantaten gemeten. Tevens werd gekeken naar de implantaatoverleving.
Tijdens de osseointegratieperiode bleken 3 implantaten verloren te zijn gegaan in de test groep (bij 2 patiënten) en geen implantaten in de controle groep, wat resulteerde in een implantaat overlevingspercentage van, respectievelijk, 91% en 100%. Na het functioneel

belasten van de implantaten zijn geen implantaten meer verloren gegaan. Eén jaar na het functioneel belasten van de implantaten bleken voorts geen verschillen tussen beide groepen te bestaan in de peri-implantaire parameters, noch werd er een verschil in röntgenologisch botverlies gezien.

In **hoofdstuk 7** worden de voornaamste onderzoeksresultaten bediscussieerd en worden conclusies getrokken. Op basis van de resultaten van de systematische literatuurstudie moge het duidelijk zijn dat het implantaatoverlevingspercentage onafhankelijk lijkt te zijn van het materiaal dat gebruikt is voor de sinusbodemelevatie chirurgie, tenminste als een voldoende lange genezingstijd in acht wordt genomen voordat de implantaten worden geplaatst (**hoofdstuk 2**). Uit de in hoofdstuk 3 beschreven studie kwam naar voren dat Piezochirugie een goed alternatief is voor het gebruik van conventioneel roterend instrumentarium t.b.v. sinusbodemelevatie chirurgie, maar geen evidente voor- of nadelen heeft t.o.v. de conventionele techniek met roterend instrumentarium (**hoofdstuk 3**). Tenslotte kan worden gemeld dat het gebruik van BioOss® gemengd met een beenmergconcentraat rijk aan MSCs een goed alternatief vormt voor een behandeling met alleen autoloog bot, waarbij een vergelijkbare genezingsperiode in acht kan worden genomen en de morbiditeit van de donorplaats kan worden verminderd (**hoofdstukken 4** en **5**). De implantaatoverleving is lager in de MSCs-groep, maar de conditie van de peri-implantaire weefsels blijkt een jaar na het functioneel belasten van de implantaten gelijk te zijn voor de toegepaste technieken (**hoofdstuk 6**). In vervolgonderzoek moet worden uitgezocht of c.q. voor welke indicaties het toepassen van BioOss® gemengd met een beenmergconcentraat verrijkt met MSCs een betrouwbaar alternatief is c.q. de voorkeur geniet boven de toepassing van autoloog bot.

Dankwoord

Dankwoord

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Curriculum Vitae

Daniela Rickert was born on September 11th in 1983 in Cologne, Germany. After finishing secondary school in 2003 at "Geschwister-Scholl-Gymnasium" in Pulheim (Germany), she immigrated to the Netherlands. There she passed the state exam for Dutch and in 2004 she started her study Dentistry at the University of Groningen. She obtained her qualification as a dentist in 2009. In may 2009 she started her PhD research project at the department of Oral and Maxillofacial Surgery of the University Medical Center Groningen. She is also working as a dentist in a private practice in Beilen.

Daniela is married to Sander Berghuis. Together they have a son, Luc, who was born on January 13th in 2011.